Applied Biosystems

GeneMapper® *ID-X* Software

Version 1.1 (Mixture Analysis Tool)

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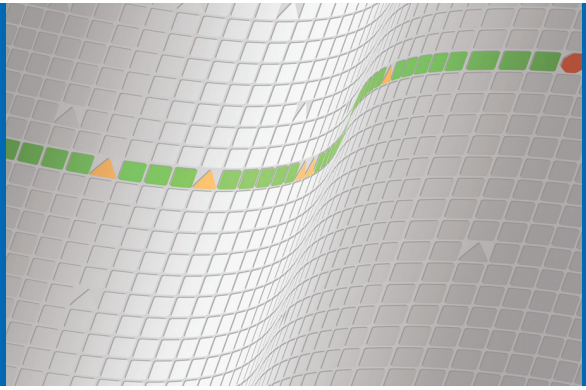
GeneMapper®
ID-X



Applied Biosystems

GeneMapper® *ID-X* Software

Version 1.1 (Mixture Analysis Tool)

1 Getting Started

2 Understanding the Software

3 Prepare for Mixture Analysis

4 Segregate Samples

5 Analyze 2-Contributor Mixtures with Two Unknowns

6 Analyze 2-Contributor Mixtures with One Known Contributor

7 Analyze Merged Results

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Preface

How to Use This Guide

Purpose of This Guide

The *GeneMapper® ID-X Software Version 1.1 Getting Started Guide* explains how to perform mixture analysis using the GeneMapper® *ID-X* Software Version 1.1. This guide functions as:

- An introduction to the mixture analysis features of GeneMapper® *ID-X* Software Version 1.1.
- A tutorial, using example experimental data provided with the GeneMapper® *ID-X* Software Version 1.1.
- A guide for your own experiments.

Audience

This guide is written for forensic analysts who perform AmpFℓSTR® kit data analysis using the GeneMapper® *ID-X* Software and who routinely perform analysis of mixture data.

Assumptions

This guide assumes that you have:

- Installed GeneMapper® *ID-X* Software Version 1.1 as described in the *GeneMapper® ID-X Software Version 1.0/1.1 Installation Guide*.
- Used AmpFℓSTR® amplification kit data for human identification (HID) applications.
- Developed a working knowledge of the Microsoft® Windows® XP operating system.

Text Conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.

- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

  Right-click the sample row, then select **View Filter ▸ View All Runs**.

User Attention Words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: – Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! – Provides information that is necessary for proper instrument or software operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: Each registered user has his or her own set of preferences. When you set these options, if affects only the user currently logged in.

IMPORTANT! To verify your client connection to the database, you need a valid user ID and password.

How to Obtain More Information

Related Documentation

The following related documents are shipped with the system:

- ***GeneMapper® ID-X Software Version 1.1 Quick Reference Guide*** – Provides a quick look at the new GeneMapper® *ID-X* Software Mixture Analysis tool features and their use.
- ***GeneMapper® ID-X Software Version 1.0/1.1 Installation Guide*** – Replaces the version 1.0 Installation Guide and provides instructions for installing the GeneMapper® *ID-X* Software v1.0 or v1.1 as a full installation, a client-only installation, or a co-installation.
- ***GeneMapper® ID-X Software Version 1.0 Getting Started Guide*** – Provides a workflow and tutorial that guides the user through the new ID-X features.
- ***GeneMapper® ID-X Software Version 1.0 Administrator's Guide*** – Provides procedures for creating user accounts, user groups, and security groups; configuring the audit trail and E-signature tools; and maintaining version 1.0 of the GeneMapper® *ID-X* Software.
- ***GeneMapper® ID-X Software Version 1.0 Quick Reference Guide*** – Provides abbreviated procedures for analyzing, viewing, and interpreting data using GeneMapper® *ID-X* Software.
- ***GeneMapper® ID-X Software Version 1.0 Reference Guide*** – Describes peak detection, sizing, and genotyping algorithms, and the GeneMapper® *ID-X* Software quality value system.
- ***GeneMapper® ID-X Software Help*** – Contains context-sensitive help for all screens and procedures and background information needed to use the software.

Portable document format (PDF) versions of this guide and the other documents listed above are also available on the *GeneMapper® ID-X Software Version 1.1 Documentation CD*.

Note: To open the user documentation included on the *GeneMapper® ID-X Software Version 1.1 Documentation CD*, use the Adobe® Acrobat® Reader® software available from **www.adobe.com**.

Note: For additional documentation, see "How to Obtain Support" on page xi.

Obtaining Information from the Help System

The GeneMapper® *ID-X* Software has a Help system that describes how to use each feature of the user interface. Access the Help system by doing one of the following:

- Click in the toolbar of the Project window
- Select **Help ▸ Contents and Index**
- Press **F1**

You can use the Help system to find topics of interest by:

- Reviewing the table of contents
- Searching for a specific topic
- Searching an alphabetized index

You can also access PDF versions of all documents in the GeneMapper® *ID-X* Software document set from the Help system.

Send Us Your Comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is only for submitting comments and suggestions relating to documentation. To order documents, download PDF files, or for help with a technical question, go to **http://www.appliedbiosystems.com**, then click the link for **Support**. (See "How to Obtain Support" on page xi).

How to Obtain Support

For HID support, you can send an e-mail to **HIDTechSupport@appliedbiosystems.com** or call **888-821-4443** option **1**.

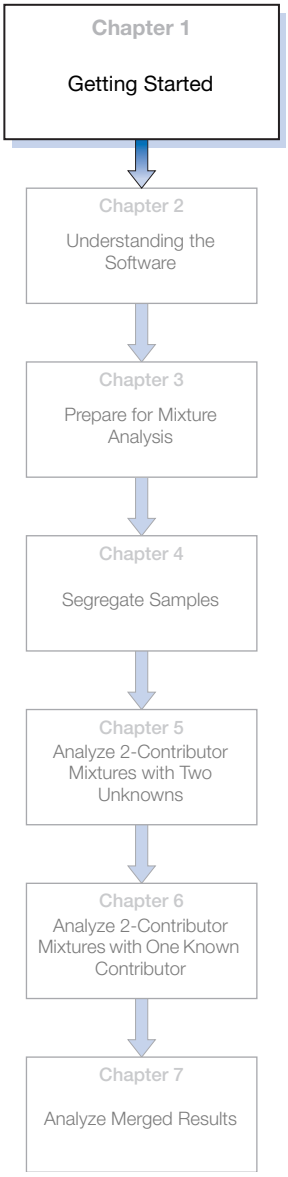
For HID support outside North America, contact your local support office.

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

1 Getting Started



This chapter covers:

- What's New in the GeneMapper® ID-X Software: The Mixture Analysis Tool 2
- GeneMapper® ID-X Software Mixture Analysis Workflows . . 4
- GeneMapper® ID-X Software Mixture Analysis Workflows . . 4
- Using this Guide for Mixture Analysis. 6

What's New in the GeneMapper® *ID-X* Software: The Mixture Analysis Tool

GeneMapper® *ID-X* Software is an automated genotyping software solution for all human identification (HID) data analysis needs, including forensic casework, databasing, and paternity testing.

GeneMapper® *ID-X* Software v1.1 contains a new Mixture Analysis Tool designed to:

- Help the forensic analyst interpret DNA mixtures.
- Evaluate autosomal DNA mixtures and 1-contributor (single-source) samples (Y-STR data cannot be evaluated using the tool).
- Save the mixture analysis results to the GeneMapper® *ID-X* Software project.

Note: The Mixture Analysis Tool supplements, but does not replace, mixture interpretation by an experienced analyst.

The Mixture Analysis Tool is accessible from the Tools menu in a GeneMapper® *ID-X* Software project. For all samples in a project, the tool performs the following functions:

- Identifies samples that are eligible for Mixture Analysis. Samples are assessed according to sample type, analysis status and allele label status.

Note: Only analyzed samples with green or yellow sizing quality (SQ) flags and no off ladder (OL)-only allele labels are eligible for mixture analysis. Unanalyzed samples, controls, allelic ladders and samples that fail sizing or have no allele labels are *not eligble*.

- Categorizes and segregates eligible samples by the minimum number of contributors contained in each sample (1, 2, or 3 or more contributors).

- Extracts and separates contributor profiles into individual major and minor genotype combinations for all markers present. (2 contributors only).
- Filters known profiles from selected sample profiles (2 contributors only).
- Calculates statistics using population databases stored in the Mixture Analysis Manager.
- Displays a ranked set of possible genotype combinations to facilitate interpretation.

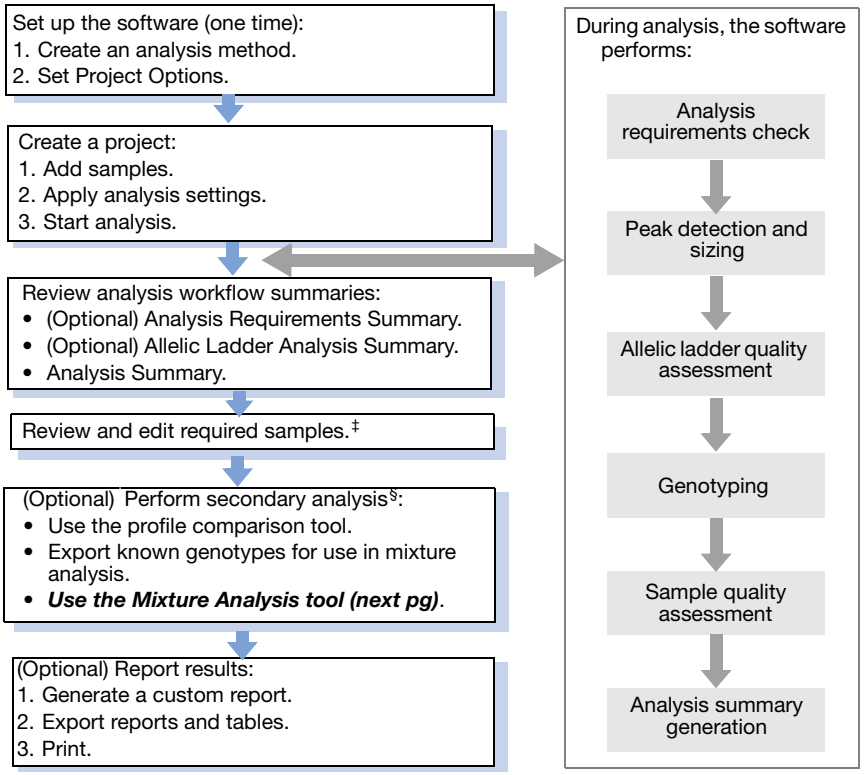
Refer to Chapter 2, Understanding the Software, for an overview of the software functions and to learn how to access the example data files for use with the Mixture Analysis Tool tutorial.

For more detailed information, refer to Appendix A, "Statistical Analysis and Algorithms," and to the *GeneMapper® ID-X Software Help*.

GeneMapper® *ID-X* Software Mixture Analysis Workflows

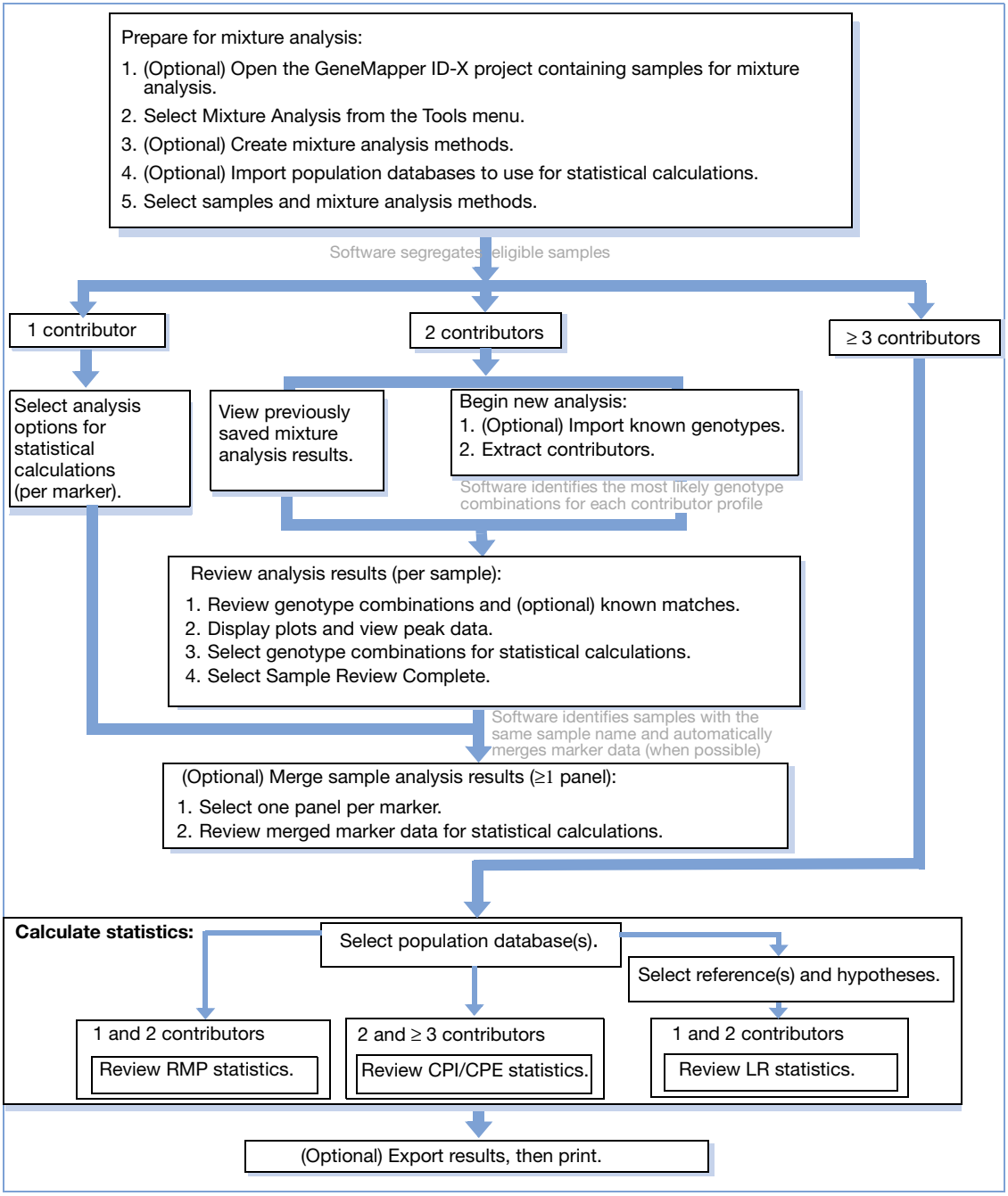
The GeneMapper® *ID-X* Software flowchart summarizes the steps for performing a typical data analysis workflow using the GeneMapper® *ID-X* Software. To the left are the steps the user performs when analyzing samples and interpreting results. To the right are the software operations that occur automatically during analysis. The Mixture Analysis flowchart that follows summarizes the steps for performing mixture analysis using the GeneMapper® *ID-X* Software.

GeneMapper® *ID-X* Software Workflow


Set up the software (one time):
1. Create an analysis method.
2. Set Project Options.
Create a project:
1. Add samples.
2. Apply analysis settings.
3. Start analysis.
Review analysis workflow summaries:
• (Optional) Analysis Requirements Summary.
• (Optional) Allelic Ladder Analysis Summary.
• Analysis Summary.
Review and edit required samples.‡
(Optional) Perform secondary analysis§:
• Use the profile comparison tool.
• Export known genotypes for use in mixture analysis.
• Use the Mixture Analysis tool (next pg).
(Optional) Report results:
1. Generate a custom report.
2. Export reports and tables.
3. Print.
During analysis, the software performs:
Analysis requirements check
Peak detection and sizing
Allelic ladder quality assessment
Genotyping
Sample quality assessment
Analysis summary generation

‡Based on your lab protocol.
§ Cannot be used for samples containing alleles with off-ladder (OL) only labels.

GeneMapper® *ID-X* Mixture Analysis Workflow


Prepare for mixture analysis:
1. (Optional) Open the GeneMapper ID-X project containing samples for mixture analysis.
2. Select Mixture Analysis from the Tools menu.
3. (Optional) Create mixture analysis methods.
4. (Optional) Import population databases to use for statistical calculations.
5. Select samples and mixture analysis methods.
Software segregates eligible samples
1 contributor
2 contributors
≥ 3 contributors
Select analysis options for statistical calculations (per marker).
View previously saved mixture analysis results.
Begin new analysis:
1. (Optional) Import known genotypes.
2. Extract contributors.
Software identifies the most likely genotype combinations for each contributor profile
Review analysis results (per sample):
1. Review genotype combinations and (optional) known matches.
2. Display plots and view peak data.
3. Select genotype combinations for statistical calculations.
4. Select Sample Review Complete.
Software identifies samples with the same sample name and automatically merges marker data (when possible)
(Optional) Merge sample analysis results (≥1 panel):
1. Select one panel per marker.
2. Review merged marker data for statistical calculations.
Calculate statistics:
Select population database(s).
Select reference(s) and hypotheses.
1 and 2 contributors
Review RMP statistics.
2 and ≥ 3 contributors
Review CPI/CPE statistics.
1 and 2 contributors
Review LR statistics.
(Optional) Export results, then print.

Using this Guide for Mixture Analysis

Overview

This guide is designed to familiarize you with the key features and functions of the GeneMapper® *ID-X* Mixture Analysis Tool and to take you through an example mixture analysis workflow. The workflow is presented in a tutorial format starting in Chapter 3, "Prepare for Mixture Analysis."

The tutorial uses the example data provided with the Mixture Analysis Tool. Refer to Chapter 2, "Understanding the Software," for information on how to access the example data. Once you complete the tutorial, you will understand how the Mixture Analysis Tool works, and you will be able to create your own settings and evaluate your own mixture and single-source data.

IMPORTANT! How you use the Mixture Analysis Tool in your laboratory and the settings you create depend on the mixture analysis and interpretation guidelines established by your internal validation process.

.

Chapter	Description
Chapter 2, Understanding the Software	• Mixture analysis methods. • Sample segregation and statistical calculations. • Population databases. • Example data
Chapter 3, Prepare for Mixture Analysis	1. Create a GeneMapper® *ID-X* Software project, add samples, and select table settings. 2. Import an analysis method. 3. Analyze data using appropriate analysis settings. 4. Review the analysis results (Analysis Requirements Summary, Analysis Summary). 5. Export and view a known genotype for later use.

Chapter	Description
Chapter 4, Segregate Samples	1. Launch the Mixture Analysis Tool. 2. Import a population database. 3. Create a new mixture analysis method. 4. Segregate the samples into one of the three classification groups.
Chapter 5, Analyze 2-Contributor Mixtures with Two Unknowns	1. Extract individual genotypes of the two possible contributors to a two-person mixture where both contributors are unknown. 2. Use the GeneMapper® *ID-X* Mixture Analysis Quality Value system to review the results of the extraction . 3. Determine which genotype combinations to include in the statistical calculations. 4. Calculate Random Match Probabilities (RMP) for each individual contributor. 5. Calculate Combined Probability of Inclusion/Exclusion (CPI/CPE) for the mixed sample. 6. Review the results of the RMP and CPI/CPE calculations. 7. Calculate and review Likelihood Ratio (LR) results.
Chapter 6, Analyze 2-Contributor Mixtures with One Known Contributor	1. Assign known genotypes. 2. Extract individual genotypes of the two possible contributors to a two-person mixture where 1 contributor is known. 3. Determine which genotype combinations to include in the statistical calculations. 4. Calculate Random Match Probabilities (RMP) for each individual contributor. 5. Calculate and review Likelihood Ratio (LR) results.
Chapter 7, Analyze Merged Results	1. Create a project containing the same sample analyzed with both the AmpFℓSTR® Profiler Plus® and COfiler® kits. 2. Add samples to the project. 3. Analyze the samples. 4. Prepare for mixture analysis. 5. Segregate and review individual profiles. 6. Merge the data to create a composite profile using the 13 genetic markers detectable from both kits. 7. Calculate statistics and review results from replicate amplifications of the same sample.

IMPORTANT! Some steps performed in this Getting Started Guide are meant only to demonstrate the use of certain features in the GeneMapper® *ID-X* Software v1.1 and may not be a part of your routine analysis workflow. Refer to your own lab procedures when using the GeneMapper® *ID-X* Software for forensic analysis.

For More Information

This guide contains basic procedures. It does not describe all the features and parameters in the GeneMapper® *ID-X* Software v1.1. For detailed information on topics presented in this guide, see the *GeneMapper® ID-X Software Help*.

Note: Before using any of the procedures in this guide, you must have successfully installed and registered the GeneMapper® *ID-X* Software v1.1. For additional information, see the *GeneMapper® ID-X Software v1.0/1.1 Installation Guide.*

Proceed to Chapter 2, "Understanding the Software," for an overview of the software functions and to learn how to access the example data files for use with the Mixture Analysis Tool tutorial.

Glossary of Terms

Term	Definition
ADBI (Above Detection, Below Interpretation)	A column in the Mixture Analysis Results Viewer - Genotype Combinations table. Also, a quality flag that indicates whether any allele peak height within a marker genotype combination falls above the minimum peak amplitude threshold set in the GeneMapper® *ID-X* Software, and above or below the Mixture Interpretation Threshold (MIT) set in the mixture analysis method.
Allele Frequency	The relative occurrence of a particular allele in a given population. In the Mixture Analysis tool, the allele frequencies associated with an individual population database stored in the Mixture Analysis Manager are used to calculate the genotype frequencies for a particular DNA profile. **Note:** Each marker within a population is associated with a sample size *(n)* used to determine the minimum allele frequency (calculated as 5/2*n*). The minimum allele frequency is automatically assigned to any allele in each marker when an allele frequency is either not observed or below the calculated minimum allele frequency (designated by *).
C1 (Major)	The DNA profile within a 2-contributor mixture sample representing the greater proportion of DNA corresponding to greater peak heights at each marker within the sample mixture. In general, for mixtures of 1:3 or higher ratios, the allele peak heights from the major contributor are clearly higher than the allele peak heights from the minor contributor. In situations where mixtures approaching 1:1 are analyzed, the major and minor contributors may become indistinguishable.
C2 (Minor)	In a 2-contributor mixture sample, the DNA profile representing the minority proportion of DNA corresponding to lower peak heights at each marker within the sample mixture. In general, for mixtures of 1:3 or higher ratios, the allele peak heights from the minor contributor are clearly lower than the allele peak heights from the major contributor, and in some cases, alleles or markers may drop out. In situations where mixtures approaching 1:1 are analyzed, the major and minor contributors may become indistinguishable.

Term	Definition
Combined Frequency	The sum of all possible genotype frequencies at a given marker when multiple possible genotypes exist. In the Mixture Analysis Results Viewer - RMP Statistics Details table, the column showing the combined genotype frequency for a given marker.
Contributor	An individual whose DNA profile is present in a mixture sample. For 2-person mixture samples in the Mixture Analysis tool, contributor 1 (major contributor) = C1 (Major), and contributor 2 (minor contributor) = C2 (Minor).
CPE (Combined Probability of Exclusion)	The probability that a random person would be excluded as a possible contributor to the observed DNA mixture. **Note:** A CPE may be calculated in instances involving 2 or more contributors to a mixture. This calculation is not available for single-source samples.
CPI (Combined Probability of Inclusion)	The probability that a random person would be included as a possible contributor to the observed DNA mixture. **Note:** A CPI may be calculated in instances involving 2 or more contributors to a mixture. This calculation is not available for single-source samples.
Extraction	The process of separating a 2-person mixture sample into individual contributor profiles and identifying the most likely genotype combinations for each contributor profile.
F Allele	An allele designation used to indicate the potential for allelic dropout. In the Mixture Analysis tool, an F allele is included in a genotype combination if detected peaks are sufficiently low that a potential heterozygous partner to one of the detected peaks could exist below the Mixture Interpretation Threshold (MIT) within the constraints of the Peak Height Ratio (PHR) settings.
Filtering	The process of identifying eligible samples in the GeneMapper® *ID-X* Software project to prevent ineligible samples from being imported into the Mixture Analysis tool.
Genotype Combination	A pair of genotypes that could represent the two individual contributors to a 2-person mixture sample.
Genotype Frequency	A column in the Mixture Analysis Results Viewer - RMP Statistics Details table. Also, the relative occurrence of a particular genotype in a given population.

Term	Definition
Genotype Profile	Allele designations for all markers of a single-source sample or an individual contributor to a mixture sample.
Heterozygote	Individual with two different alleles at a particular marker (locus).
Homozygote	Individual with one allele at a particular marker (locus).
Hypothesis	See Likelihood Ratio.
Inconclusive	A designation given to a marker for which the genotype cannot be determined with certainty. In the Mixture Analysis tool, inconclusive markers are not included in statistical analysis.
IQ (Inclusion Quality)	A column in the Mixture Analysis Results Viewer - Genotype Combinations tables. Also, a composite quality flag that indicates the Peak Height Ratio (PHR) Status and the Residual Status for genotype combinations.
Known Filtering	The process whereby a known genotype is used to reduce (filter) the list of genotype combinations extracted from a 2-person mixture sample to display only those combinations that also match the known genotype profile. In the Mixture Analysis tool, the genotype combinations of the contributor with the best match to the known contributor are displayed in the Genotype Combinations tables of the Mixture Analysis Results Viewer. Where a known genotype is used during the extraction process, only the Selected Genotype Combinations associated with the unknown contributor to the mixture sample are included in statistical analysis.
Known Genotype Profile	Genotype of a reference sample used for comparison to a mixture sample where a known genotype is inferred (for example, an intimate body swab sample). In the Mixture Analysis tool, the known genotype profile is matched to one of the contributor profiles extracted from a 2-person mixture sample, and is used to filter the genotype combinations tables to display only those combinations that contain the known contributor.

Term	Definition
Known Match	An exact match of the known genotype to one of the contributors extracted from a 2-person mixture sample. In the Mixture Analysis tool, statistical analysis can only be performed on the unknown contributor when there is a complete (100%) match of the known genotype to a single contributor, either C1 (Major) or C2 (Minor), as displayed in the Selected Genotype Combinations table of the Mixture Analysis Results Viewer.
Known Matching	The process whereby a known genotype profile is compared to both of the contributor profiles extracted from a 2-person mixture sample to determine which contributor displays the best match to the known.
LR (Likelihood Ratio)	A ratio of the probabilities of two hypotheses that offer different explanations for the existence of the DNA profile evidence (e.g. possible contributors to the mixture sample). **Note:** In the Mixture Analysis tool, LR statistical calculations are only performed on 1-contributor and 2-contributor samples.
Major Contributor	See C1 (Major)
Marker Inclusion Frequency	In the Mixture Analysis Results Viewer - CPI/CPE Statistics Details table, the column showing the probability that a random person would be included as a possible contributor to the observed DNA mixture at a given marker.
Minimum Allele Frequency	Value used in statistical analysis of DNA profiles representing either alleles not present in the population database or alleles that have an observed allele frequency below the calculated minimum allele frequency. Calculated using the following formula: Minimum allele frequency = $5/2n$ where n = number of samples for each marker in the ethnic population.
Minor Contributor	See C2 (Minor).
Missing Markers	Markers that are present in the mixture sample, but are not represented in the Selected Genotype Combinations table or the known genotype profile.
MIT (Mixture Interpretation Threshold)	A user-defined setting in the mixture analysis method. Also, the minimum peak height threshold used for mixture analysis.

Term	Definition
Mixture	Sample containing DNA from two or more contributors.
Mixture Analysis	The process of identifying the minimum number of possible contributors to a mixture sample from data produced using a single AmpFℓSTR® panel, extracting the potential genotypes of the contributors (2-person mixtures only), and performing statistical analysis.
Mixture Analysis (Merged Results)	The process of identifying the minimum number of possible contributors to a mixture sample from data produced using one or more AmpFℓSTR® panels, extracting the potential genotypes of the contributors (2-person mixtures only), and performing statistical analysis on a composite profile generated from replicate results from the same sample or from results generated from the same sample using different AmpFℓSTR® kits.
Mixture Analysis Method	A collection of user-defined settings that determine the sample segregation and extraction thresholds used by the Mixture Analysis tool to analyze potential mixture samples transferred from a GeneMapper® *ID-X* Software project.
Mixture Analysis Parameters	The heterozygote Peak Height Ratio (PHR) settings and Mixture Interpretation Threshold (MIT) as defined in the mixture analysis method, and used to perform sample segregation and extraction on selected mixture samples within the Mixture Analysis tool.
Mixture Analysis Project	The mixture analysis results for a group of samples transferred into the Mixture Analysis tool from a GeneMapper® *ID-X* Software project.
Mixture Analysis Tool	Part of the GeneMapper® *ID-X* Software containing specific functionality to assist in the analysis, interpretation and statistical analysis of DNA mixtures.
Mx (Mixture Proportion)	A measure of the relative proportion of the minor contributor in a 2-person mixture sample, as described by Gill, *et al*., 1998 (see Bibliography).

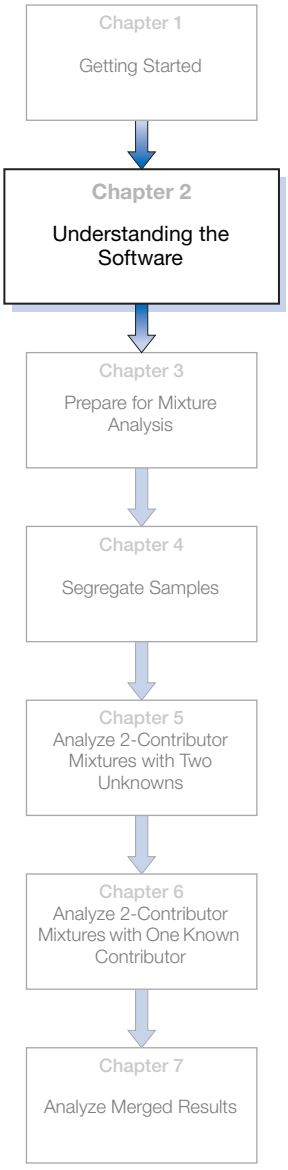
Term	Definition
PHR Status	A column in the Mixture Analysis Results Viewer - Genotype Combinations tables. Also, a mixture analysis quality flag that indicates whether the calculated Peak Height Ratio (PHR) for a given genotype combination falls above or below the Peak Height Ratio (PHR) thresholds set per peak height range in the mixture analysis method. **Note:** The PHR thresholds defined in a mixture analysis method and applied to samples in the Mixture Analysis tool differ from the minimum peak height ratio threshold defined in an analysis method and applied to samples in a GeneMapper® *ID-X* Software project.
Population Database	A collection of the alleles and allele frequencies obtained from a random group of unrelated individuals from one or more ethnic groups. In the Mixture Analysis tool, these allele frequencies are used to calculate genotype frequencies for a DNA profile. **Note:** Each marker within a population is associated with a sample size (*n*) used to determine the minimum allele frequency (calculated as 5/2*n*). The minimum allele frequency is automatically assigned to any allele in each marker when an allele frequency is either not observed or below the calculated minimum allele frequency (designated by *).
Profile (Sample)	The genotype (allele designations) of a sample. In the Mixture Analysis tool, you can import known profiles into a mixture analysis project to compare against each of the contributor profiles extracted from a 2-person mixture sample as part of mixture interpretation.
Profile Frequency	An entry in the Mixture Analysis Results Viewer - RMP Statistics Summary section. The estimated frequency of occurrence of a particular profile based on values from a given population database.
Reference Profile	In the Profile Comparison tool, the profile against which another profile is compared to determine the % Match. The software performs pairwise comparisons to determine the direction of comparison that yields the higher % Match, then reports only the direction of comparison with the higher % Match. In the Mixture Analysis tool, one or two reference profiles (known genotypes) can be assigned to a mixture sample when calculating Likelihood Ratio (LR) statistics.

Term	Definition
Residual	A measure of how close the observed contributor proportions for a particular genotype combination are to the expected contributor proportions for a particular 2-person mixture sample, as described by Gill, *et al*., 1998 (see Bibliography).
Residual Status	A column in the Mixture Analysis Results Viewer - Genotype Combinations tables. It indicates whether the calculated residual value for a genotype combination falls above or below the residual threshold of 0.04 supplied with the Mixture Analysis tool.
Residual Threshold	As defined in the Mixture Analysis tool, the value (0.04) above which genotype combinations are not automatically considered as possible contributors to the mixture sample. **Note:** The residual threshold, set internally within the Mixture Analysis tool based on extensive testing of DNA mixtures, cannot be adjusted by the user.
RMP (Random Match Probability)	The probability that an individual chosen at random from the population has a DNA profile that matches the profile being compared. In the Mixture Analysis tool, RMP statistical calculations are only performed on 1-contributor and 2-contributor samples.
Sample Segregation	The process by which samples transferred into the Mixture Analysis tool from a GeneMapper® *ID-X* Software project are identified as containing a minimum of 1, 2, or 3 or more contributors and separated into the appropriate mixture analysis workflow for each contributor category.
Sample Selection	The process by which potential mixture samples transferred into the Mixture Analysis tool from a GeneMapper® *ID-X* Software project are selected and mixture analysis methods applied in order to proceed with sample segregation.
Selected Genotype Combinations Table	Table containing genotype combinations that are included in statistical analysis. Genotype combinations are assigned to this table automatically if they are assigned a passing ( ) Inclusion Quality (IQ) during extraction of contributors.

Term	Definition
Single-Source Sample	In the Mixture Analysis tool, samples originating from only 1 contributor. Defined as samples that contain: • No markers that fail the peak height ratio (PHR) thresholds specified in the mixture analysis method *and* • Only one marker with three called alleles Random Match Probability and Likelihood Ratio calculations can be performed on single-source samples following sample segregation.
Statistical Analysis	The process of calculating statistics (Random Match Probability, Combined Probability of Inclusion, Combined Probability of Exclusion, Likelihood Ratio) for a DNA profile. **Note:** The Mixture Analysis tool excludes the Amelogenin (AMEL) marker from all statistical analyses.
Statistical Analysis Options (1 Contributor)	Column in the Mixture Analysis Results Viewer: Minimum Number of Contributors = 1 ▸ 1 Contributor Results tab. Displays the genotype frequency calculation options available for use in Random Match Probability (RMP) statistical analysis of 1-contributor samples. **Note:** The Mixture Analysis tool excludes the Amelogenin (AMEL) marker from all statistical analyses (RMP, CPI/CPE, LR). The following marker-specific genotype frequency calculation options are available, based on allele number: • One allele: Use Alleles (*Default*), Use 2p, Inconclusive • Two alleles: Use Alleles (*Default*), Inconclusive • Three alleles: Min Genotype Freq (*Default*), Inconclusive Where: • Use Alleles = Calculate the genotype frequency from the allele frequencies (use heterozygous equation [$2pq$] or homozygous equation [$p^2 + p(1\text{-}p)\theta$]) • Use 2p = Calculate the genotype frequency from the allele frequency assuming possible allelic drop-out (use conservative frequency equation [$2p$]) • Inconclusive = Do not calculate a genotype frequency for the marker (consider marker as uninformative) • Min Genotype Freq = Calculate the genotype frequency from the minimum genotype frequency for a tri-allelic marker (use $3/n$, where n = number of samples for each marker in the ethnic population as specified in the selected population database)

Term	Definition
Theta	A correction factor applied to the homozygous genotype frequency calculation that compensates for possible population substructure that may lead to an underestimate of the genotype frequency for the marker. In the Mixture Analysis tool, a user-defined setting (Population Database Selection dialog box) for each ethnic group within a population database, designated by the symbol θ.
Unselected Genotype Combinations Table	Contains genotype combinations that will not be included in statistical analysis. Genotype combinations are assigned to this table automatically if they were not assigned a passing ( ) Inclusion Quality (IQ) during extraction of contributors.

2 Understanding the Software



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- Sample Segregation and Statistical Calculations 21
- Population Databases 22
- Example Data 22

Overview

About the Software

The software performs a number of functions that require setup by the user. This chapter describes these functions.

In This Chapter

In this chapter, you will learn how the software:

- Uses mixture analysis methods.
- Segregates samples and calculates statistics.
- Uses population databases for statistical calculations.
- Uses example data to demonstrate the functionality and features of the Mixture Analysis Tool.

Mixture Analysis Methods

A mixture analysis method contains Peak Height Ratio and Mixture Interpretation Threshold information. The software uses this information to:

- Segregate samples according to their contributor categories (1, 2, or 3 or more contributors)
- Determine the most likely genotypes of the individual contributors to a 2-person mixture during the extraction process.

You will learn how to create a mixture analysis method in Chapter 4, Segregate Samples.

Note: Applied Biosystems recommends creating a set of mixture analysis methods that corresponds to the interpretation criteria developed by your laboratory based on internal validation studies. Different mixture analysis parameters may be appropriate for data generated using different AmpFℓSTR® kits, instrument platforms, or for particular sample types.

Sample Segregation and Statistical Calculations

The software segregates samples on the basis of the minimum number of contributors according to the following rules:

- **1 Contributor (single-source)** - Samples that:
  - Do not contain markers that fail the peak height ratio thresholds specified in the mixture analysis method

    and
  - Contain a maximum of 1 marker with three called alleles

  are categorized as originating from a single source.
- **2 Contributors** - Samples that contain:
  - 1 or more 2 peak markers failing peak height ratio thresholds
  - 3 or more alleles at 2 or more markers with the maximum number of alleles not exceeding 4

  are categorized as originating from a minimum of 2 contributors.
- **3 or more Contributors** - Samples that contain 1 or more markers with more than 4 alleles are categorized as originating from a minimum of 3 contributors.

The following table details the statistical options available for each of the three categories of samples:

	1 Contributor (Single-source)	2 Contributors	3 or More Contributors
Random Match Probability (RMP)	X	X	
Combined Probability of Inclusion/Exclusion (CPI/CPE)		X	X
Likelihood Ratio (LR)	X	X	

For additional information, refer to Appendix A, "Statistical Analysis and Algorithms," and to the *GeneMapper® ID-X Software Help*.

Population Databases

For the software to perform statistical calculations, it requires population databases that contain the appropriate allele frequency information. Three ready-to-use population databases are provided by Applied Biosystems to use with the Mixture Analysis Tool. You can import these databases and/or create and import custom databases based on your own allele frequency data.

Refer to Chapter 4, Segregate Samples, for information on how to import a ready-to-use population database.

To create a custom population database, refer to the *GeneMapper® ID-X Software Help*.

Example Data

Overview

Example data (.fsa files) are installed with the GeneMapper® *ID-X* Software v1.1 for use with the mixture analysis tutorial.

- SGM Plus® Kit example data are used to demonstrate features of the Mixture Analysis Tool in Chapter 3 through Chapter 6.
- COfiler® and Profiler Plus® Kit example data are used to demonstrate additional functionality of the Mixture Analysis Tool in Chapter 7.

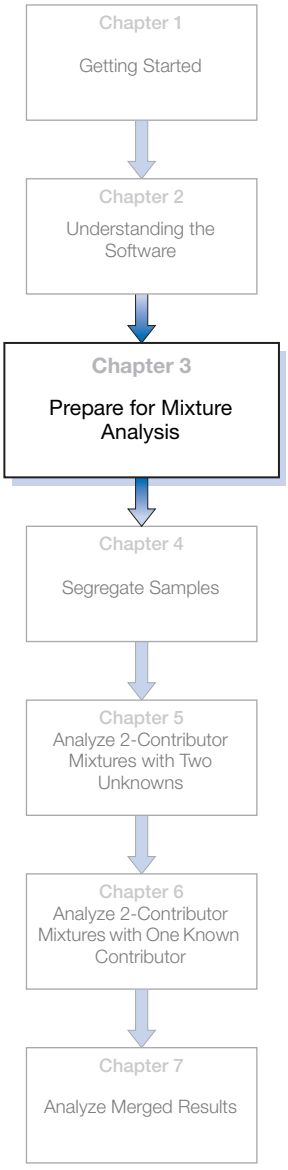
How to Access the Example Data Files

To perform the exercises described in this guide, locate the example data files provided, as shown below.

Install Configuration	File Location
	Mixture Analysis Sample Files (.fsa)
Client install	<*drive*>:\AppliedBiosystems\GeneMapperID-X\Client\Example Data\Mixture Analysis Samples
Full install	<*drive*>:\AppliedBiosystems\GeneMapperID-X\Example Data\Mixture Analysis Samples

Note: The drive varies depending on the installation of the GeneMapper® *ID-X* Software. See the *GeneMapper® ID-X Software Version 1.0/1.1 Installation Guide* for more information on installation options.

3 Prepare for Mixture Analysis



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- Step 2: Import an Analysis Method 28
- Step 3: Analyze Data Using Appropriate Settings 29
- Step 4: Review Analysis Results 31
- Step 5: Export and View a Known Genotype Profile 35

Overview

This chapter provides a tutorial that proceeds through the steps to prepare samples for mixture analysis within a project . You will create a GeneMapper® *ID-X* Software project as described in the mixture analysis workflow, and perform data analysis and allele editing before selecting and launching the Mixture Analysis Tool from the Tools menu (Chapter 4). You will also export a known genotype profile during this process.

Mixture Analysis Preparation

To prepare the data for mixture analysis, analyze and edit the data in a GeneMapper® *ID-X* Software project, then process the data using the Mixture Analysis Tool.

IMPORTANT! The settings used for this Getting Started Guide are for demonstration purposes only and require customization and optimization for data generated in your laboratory.

Note: Before using the procedures in this chapter you must successfully install and register GeneMapper® *ID-X* Software v1.1. For additional information, see the *GeneMapper® ID-X Software v1.0/1.1 Installation Guide*.

In This Chapter

In this chapter, you will learn how to:

- Create a GeneMapper® *ID-X* Software project, add samples, and select table settings.
- Import an analysis method.
- Analyze data using appropriate analysis settings.
- Review the analysis results (Analysis Requirements Summary, Analysis Summary).
- Export and view a known genotype profile. This profile will be used later in the analysis of mixed samples in which 1 contributor is known (for example, from an intimate body swab).

Step 1: Create a GeneMapper® *ID-X* Software Project

Open and Create a New GeneMapper® *ID-X* Project

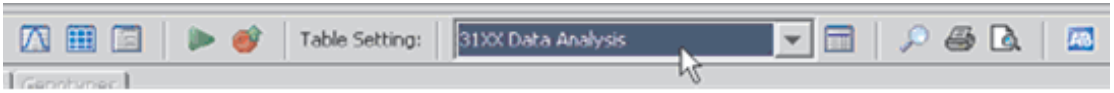
If a project is already open in the Project window, go to **File ▸ New Project** or click (New Project) to open a blank Project window.

Note: If you have a previous project open you may be prompted to save changes. Click **Yes** to save the changes.

Add Samples to the Project

1. In the Project window, Select **Edit ▸ Add Samples to Project (Ctrl-K)**.
2. Navigate to <*drive*>:\AppliedBiosystems\GeneMapper ID-X\Example Data\Mixture Analysis and select **MA_SGM**.
3. Click **Add to List** in the lower left-hand side of the window to add the sample files to the project, then click **Add** to close the dialog box.

   From the Table Setting drop down list, select **31XX Data Analysis**.



Step 2: Import an Analysis Method

Analysis methods define the peak detection, sizing, genotyping, and quality assessment parameters applied during analysis of sample data. In this step, you will import the analysis method configured specifically for use with the GeneMapper® *ID-X* Software example data provided and with the procedures outlined in this guide.

IMPORTANT! The values used in this guide may not be suitable for analyzing data generated in your laboratory. You must optimize and validate these values as part of an internal validation study.

In the GeneMapper *ID-X* Project window:

1. Select **Tools ▸ GeneMapper® ID-X Manager**.
2. In the **Analysis Methods** tab, select **Import**.
3. Navigate to <*drive*>:\AppliedBiosystems\GeneMapper ID-X\Example Data\Mixture Analysis\GSG Analysis Method and select **HID_Full Range_75**.
4. Click **OK** in the Choose Security Group dialog box to confirm the GeneMapper *ID-X* Security Group, then click **Done.**

Step 3: Analyze Data Using Appropriate Settings

1. Define each sample from left to right, as shown in the Samples table below:

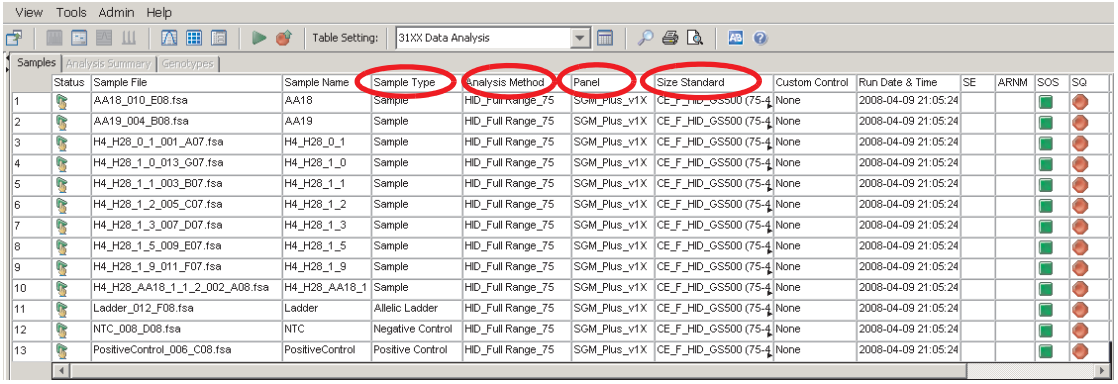
   a. Assign the Sample Type to each sample.

   b. Select the Analysis method (HID_Full Range_75).

   c. Select the Panel (SGM_Plus_v1X).

   d. Select the Size Standard ((CE_F_HID_GS500 (75-450)).

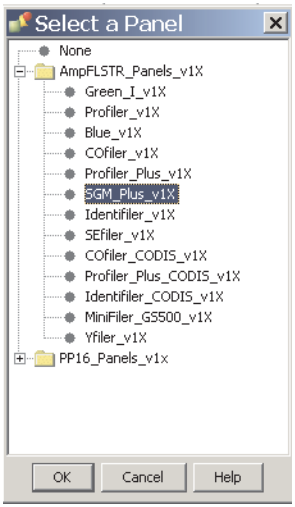
   To make your selections, click in each cell and select from the drop-down list. To change an entire column, click in the first cell, make your selection, then click in the column header and press **Ctrl-D** to populate (fill down) the column.



	Status	Sample File	Sample Name	Sample Type	Analysis Method	Panel	Size Standard	Custom Control	Run Date & Time	SE	ARNM	SOS	SQ
1		AA18_010_E08.fsa	AA18	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 21:05:24				
2		AA19_004_B08.fsa	AA19	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 21:05:24				
3		H4_H28_0_1_001_A07.fsa	H4_H28_0_1	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 21:05:24				
4		H4_H28_1_0_013_G07.fsa	H4_H28_1_0	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 21:05:24				
5		H4_H28_1_1_003_B07.fsa	H4_H28_1_1	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 21:05:24				
6		H4_H28_1_2_005_C07.fsa	H4_H28_1_2	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 21:05:24				
7		H4_H28_1_3_007_D07.fsa	H4_H28_1_3	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 21:05:24				
8		H4_H28_1_5_009_E07.fsa	H4_H28_1_5	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 21:05:24				
9		H4_H28_1_9_011_F07.fsa	H4_H28_1_9	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 21:05:24				
10		H4_H28_AA18_1_1_2_002_A08.fsa	H4_H28_AA18_1	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 21:05:24				
11		Ladder_012_F08.fsa	Ladder	Allelic Ladder	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 21:05:24				
12		NTC_008_D08.fsa	NTC	Negative Control	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 21:05:24				
13		PositiveControl_006_C08.fsa	PositiveControl	Positive Control	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 21:05:24				

Note: The green ( ) arrow in the status column indicates that these samples have never been analyzed. Unanalyzed samples will not be imported into the Mixture Analysis tool.

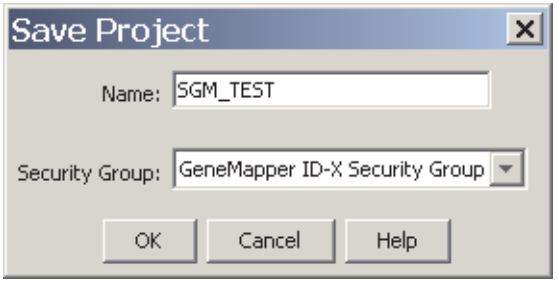
Note: When you make changes to the Panel column, a separate window opens with a folder displayed instead of a drop-down list. Open the folder and select the appropriate panel.



2. Click the **Analyze** icon on the toolbar.

The Save Project dialog box is displayed.

3. Fill in the Save Project dialog box as shown below and click **OK**.

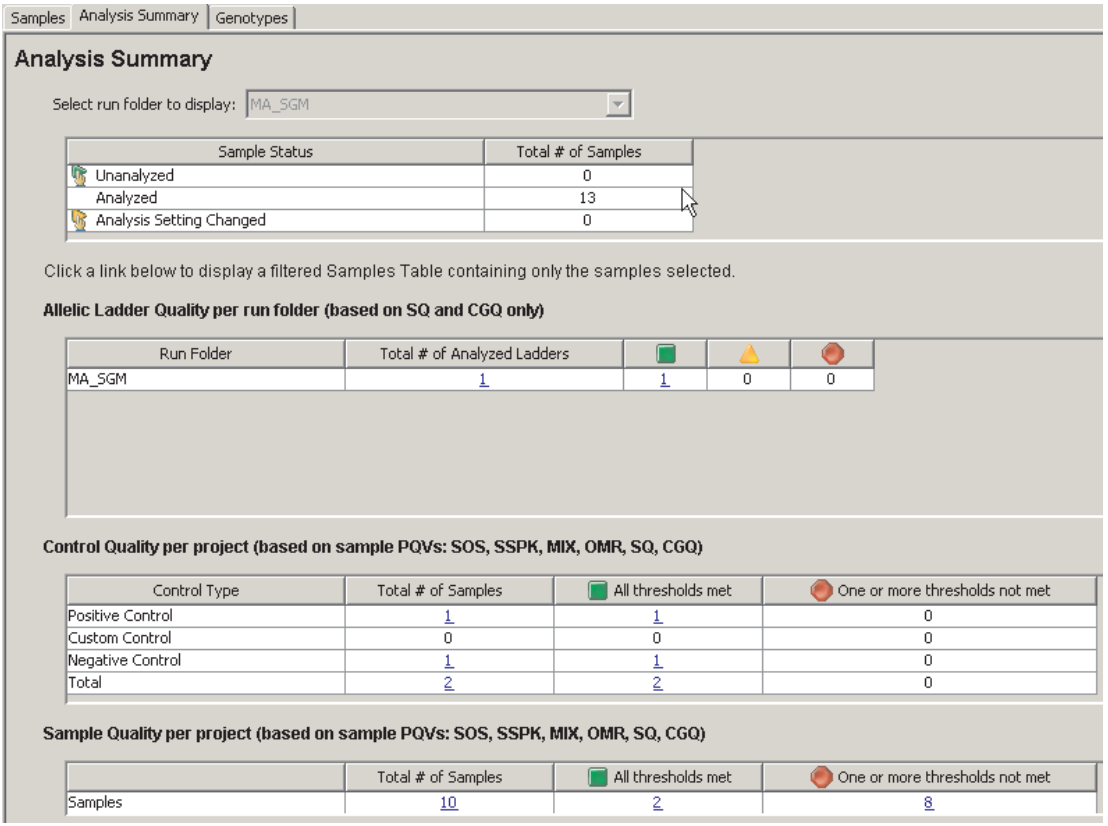


The Analysis Summary dialog box appears after analysis is completed.

Step 4: Review Analysis Results

Review Analysis Summaries

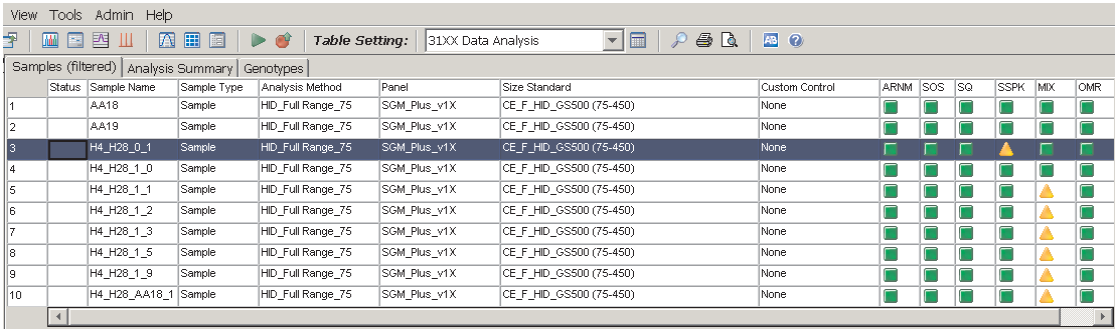
The Analysis Summary dialog box appears as shown below.



Note: If you did not set up the Samples tab in the GeneMapper *ID-X* Project window correctly, the Analysis Requirements Summary may display first. Review the reason(s) why the analysis requirements are not met and correct them to proceed with the analysis. For additional information, refer to the *GeneMapper® ID-X Software Help*.

To review the data:

1. In the Analysis Summary under Sample Quality per project, select the **10** from the Total # of Samples column (shown above) to display a filtered Samples Table that allows access to all the samples for editing and review.

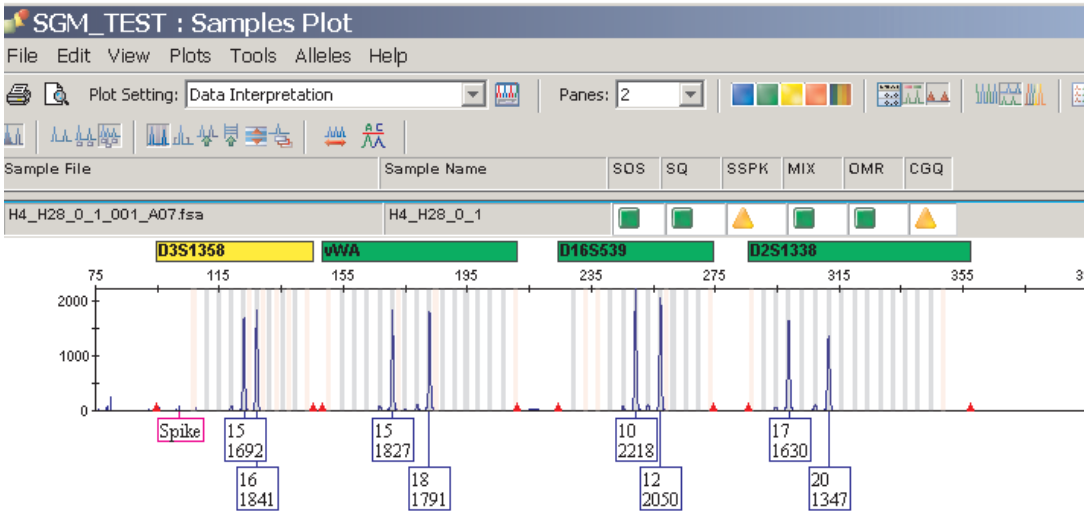


	Status	Sample Name	Sample Type	Analysis Method	Panel	Size Standard	Custom Control	ARNM	SOS	SQ	SSPK	MIX	OMR
1		AA18	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-450)	None						
2		AA19	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-450)	None						
3		H4_H28_0_1	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-450)	None						
4		H4_H28_1_0	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-450)	None						
5		H4_H28_1_1	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-450)	None						
6		H4_H28_1_2	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-450)	None						
7		H4_H28_1_3	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-450)	None						
8		H4_H28_1_5	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-450)	None						
9		H4_H28_1_9	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-450)	None						
10		H4_H28_AA18_1	Sample	HID_Full Range_75	SGM_Plus_v1X	CE_F_HID_GS500 (75-450)	None						

Note: The MIX PQV flag is yellow for many of the samples indicating that GeneMapper® *ID-X* Software has identified the samples as potential mixtures.

2. In the Samples (filtered) tab, select sample H4_H28_0_1 as shown previously, and click the display plots icon in the tool bar.

3. Select the **Data Interpretation** Plot Setting. Note the spike in the D3S1358 analysis range.



4. Highlight the **Spike** label and right-click to view the Peak Raw Data, then confirm that the peak is a spike.

Note: Peaks with magenta artifact labels such as Spike peaks will not be interpreted by the Mixture Analysis Tool.

5. Close the Samples Plot.

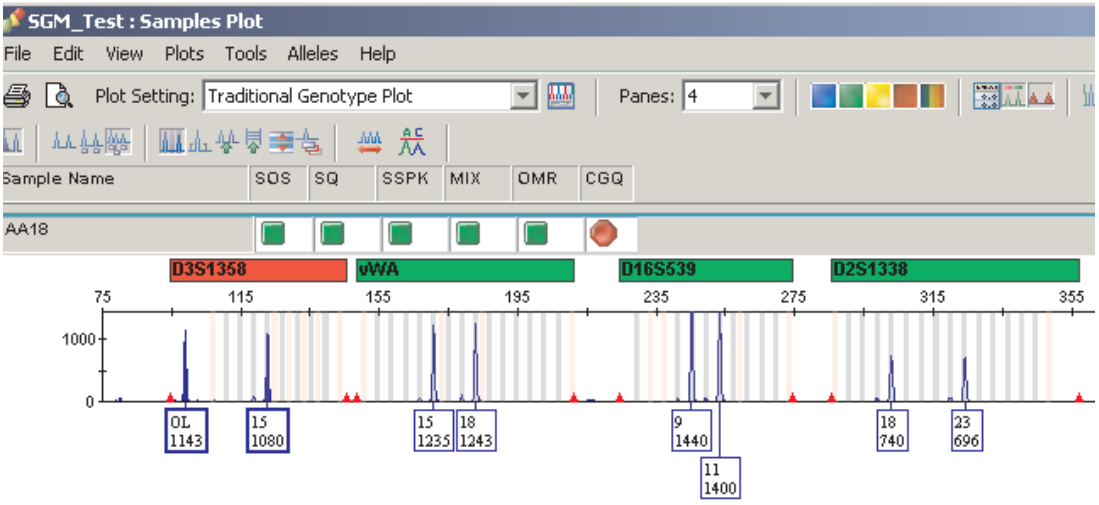
6. Highlight all sample rows and click the Display Plots icon in the tool bar.

Review and Save Samples

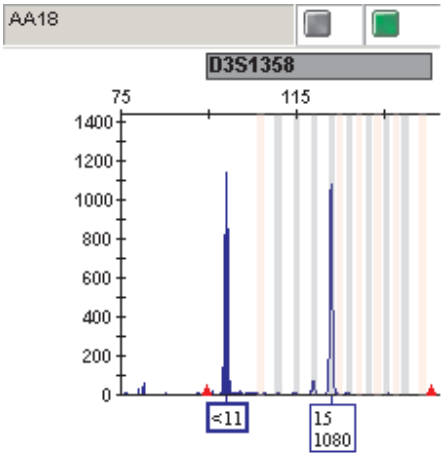
1. Review all samples to ensure that no off ladder (OL) only calls are present. Select the **Traditional Genotype Plot** Setting for review.

Note: Samples containing OL-only allele calls cannot be processed by the Mixture Analysis Tool.

Sample AA18 contains a true D3S1358 peak that is labeled as an OL allele.



2. Select, then right click on the peak label to assign a custom allele label to the peak. Label the peak as <11 as shown in the following figure. Enter a reason for change when prompted.



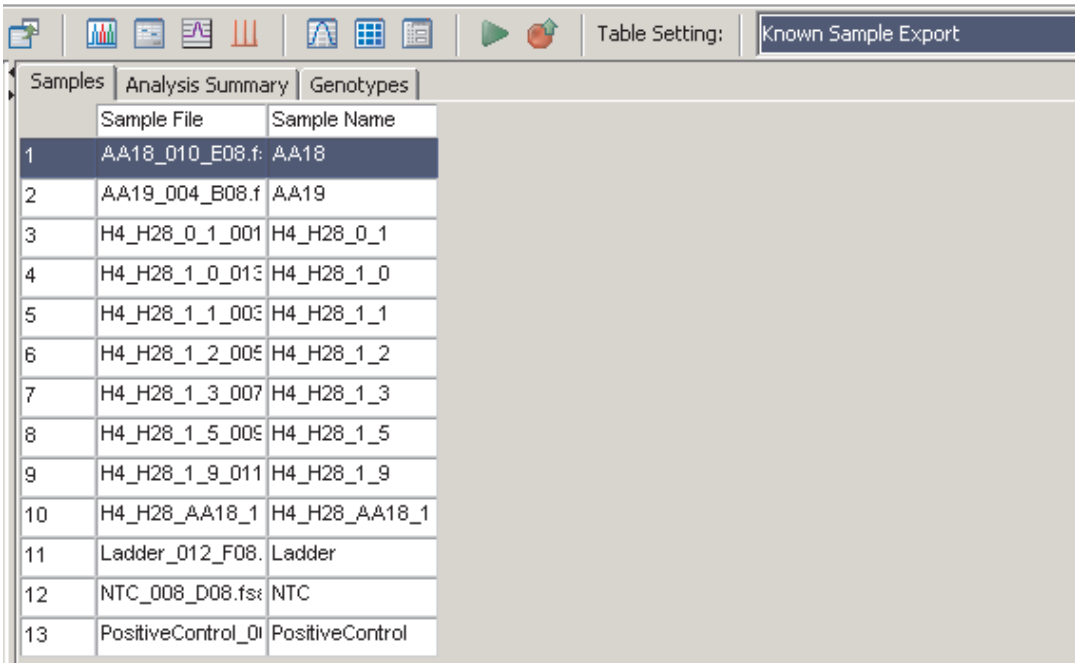
3. Repeat the labeling of the off-ladder D3S1358 allele for sample H4_H28_AA18_1_1_2.

4. When the review is complete, exit the Samples Plot.

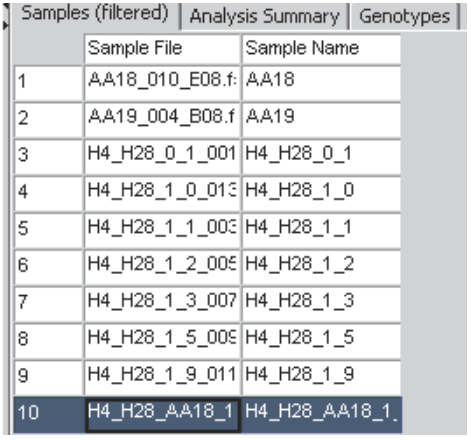
Step 5: Export and View a Known Genotype Profile

Select Samples for Viewing

1. In the Samples table, select the **Known Sample Export** Table Setting.



The Samples table should now contain only the Sample File and Sample Name columns, as shown next.



2. Select the **Genotypes** tab, which should now display only the Sample File, Sample Name, Marker, Allele 1 and Allele 2 columns, as shown.

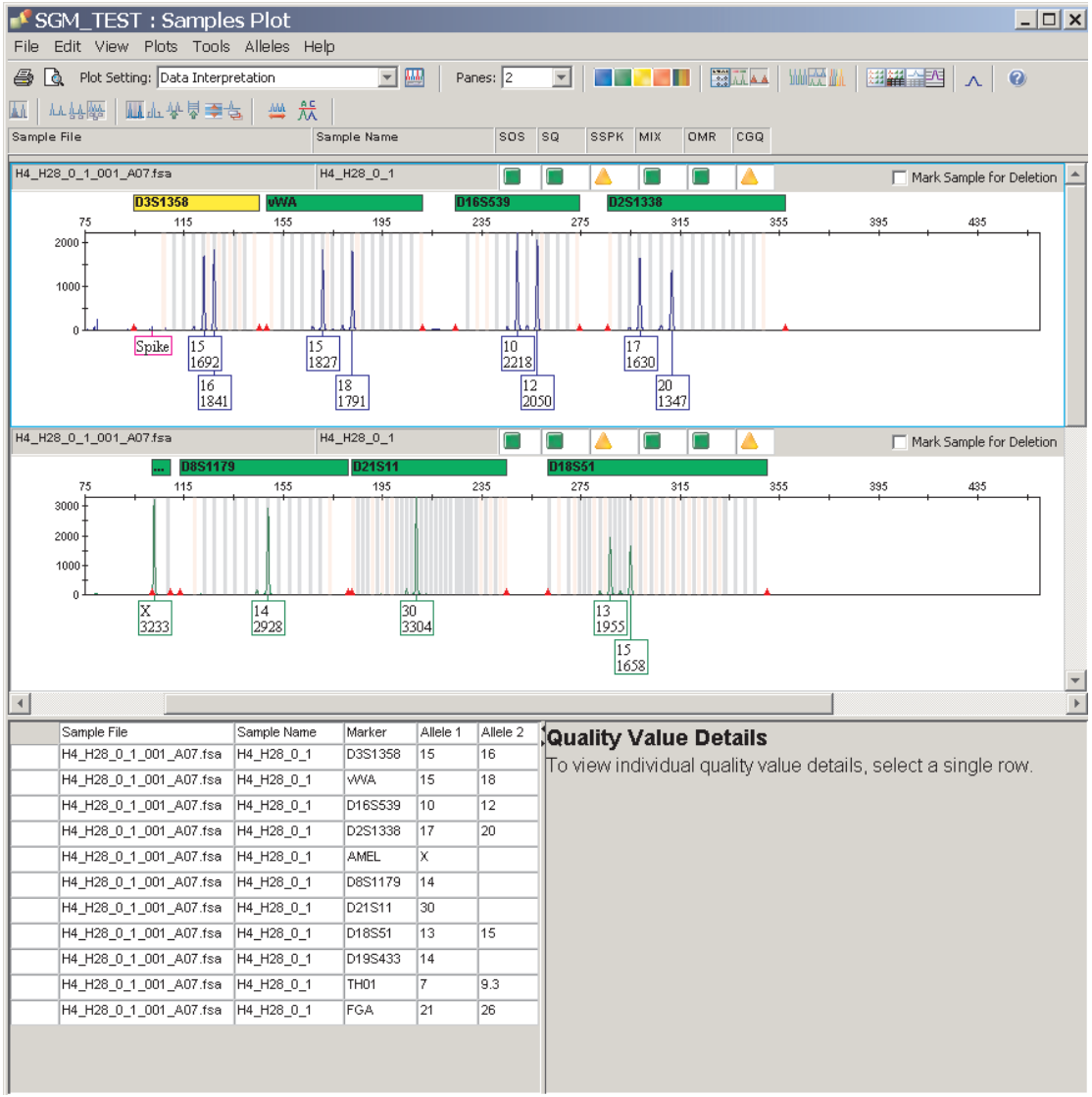
Samples (filtered) | Analysis Summary | Genotypes

	Sample File	Sample Name	Marker	Allele 1	Allele 2
1	AA18_010_E08.fsa	AA18	D3S1358	<11	15
2	AA18_010_E08.fsa	AA18	vWA	15	18
3	AA18_010_E08.fsa	AA18	D16S539	9	11
4	AA18_010_E08.fsa	AA18	D2S1338	18	23
5	AA18_010_E08.fsa	AA18	AMEL	X	Y
6	AA18_010_E08.fsa	AA18	D8S1179	14	15
7	AA18_010_E08.fsa	AA18	D21S11	28	31.2
8	AA18_010_E08.fsa	AA18	D18S51	17	20
9	AA18_010_E08.fsa	AA18	D19S433	11	12.2
10	AA18_010_E08.fsa	AA18	TH01	7	8
11	AA18_010_E08.fsa	AA18	FGA	21	22
12	AA19_004_B08.fsa	AA19	D3S1358	14	15
13	AA19_004_B08.fsa	AA19	vWA	15	16
14	AA19_004_B08.fsa	AA19	D16S539	12	
15	AA19_004_B08.fsa	AA19	D2S1338	19	
16	AA19_004_B08.fsa	AA19	AMEL	X	Y
17	AA19_004_B08.fsa	AA19	D8S1179	13	15
18	AA19_004_B08.fsa	AA19	D21S11	31	
19	AA19_004_B08.fsa	AA19	D18S51	17	
20	AA19_004_B08.fsa	AA19	D19S433	14	15
21	AA19_004_B08.fsa	AA19	TH01	8	
22	AA19_004_B08.fsa	AA19	FGA	24	25

IMPORTANT! Be sure that no off-ladder (OL)-only allele labels are present. OL alleles are not incorporated into known filtering. Allele labels containing OL are acceptable if OL is followed by another character (for example, OL < 6).

3. Select the **Samples** tab, select single-source sample **H4_H28_0_1**, then click the display plots icon in the toolbar to display the Samples Plot.

4. Select the **Data Interpretation** Plot Setting to display the Genotypes table together with the electropherogram.

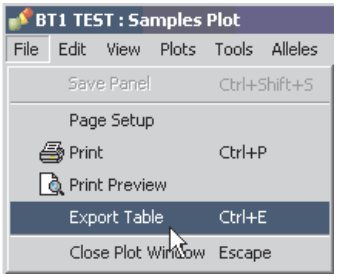


Sample File	Sample Name	Marker	Allele 1	Allele 2
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	D3S1358	15	16
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	vWA	15	18
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	D16S539	10	12
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	D2S1338	17	20
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	AMEL	X	
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	D8S1179	14	
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	D21S11	30	
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	D18S51	13	15
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	D19S433	14	
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	TH01	7	9.3
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	FGA	21	26

5. Select **File** ▸ **Export Table** (Ctrl-E).

Export the Table

1. Select **File ▸ Export Table** (Ctrl-E).



2. Select **Export File As Tab-delimited text (.txt)** file.

Note: You can export samples from the GeneMapper® *ID-X* Software as tab-delimited text files (*.txt) or as comma-separated values files (*.csv).

3. Name the file *Known_H28.txt.*

4. Choose Export destination <*drive*>:\AppliedBiosystems\GeneMapperID-X\Mixture Analysis, then click **Export**.

Note: During the routine workflow, you may prefer to export known genotypes to the run folder containing the sample file associated with the known profile.

5. Navigate to <*drive*>:\AppliedBiosystems\GeneMapperID-X\Mixture Analysis and open the exported file using Excel to view the exported data shown below.

Sample File	Sample Name	Marker	Allele 1	Allele 2
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	D3S1358	15	16
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	vWA	15	18
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	D16S539	10	12
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	D2S1338	17	20
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	AMEL	X	
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	D8S1179	14	
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	D21S11	30	
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	D18S51	13	15
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	D19S433	14	
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	TH01	7	9.3
H4_H28_0_1_001_A07.fsa	H4_H28_0_1	FGA	21	26

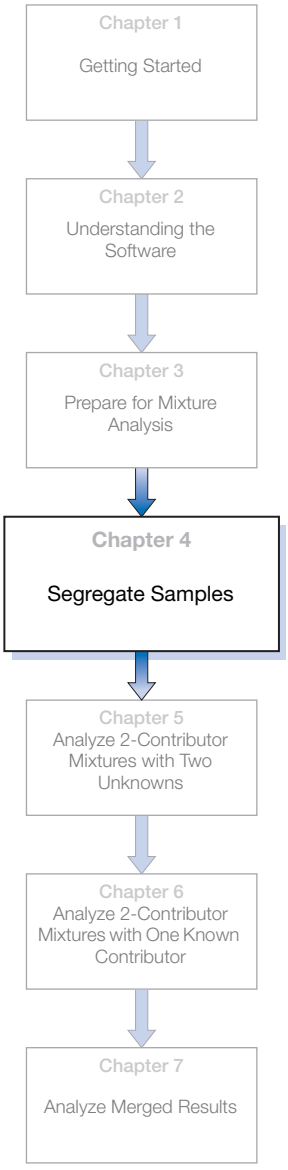
6. Return to the GeneMapper *ID-X* Project window, select the **Samples** tab, and in the Samples table select-single source sample H4_H28_1_0, then display the Samples Plot using the display plots icon in the toolbar.

7. Repeat "Select Samples for Viewing" step 4 on page 37 through "Export the Table" step 5 to export this known sample as *Known_H4.txt*.

   The exported known samples will be used later during mixture extraction and Likelihood Ratio calculations.

8. Close the Samples Plot.

4 Segregate Samples



This chapter covers:

- Overview. 42
- Step 1: Launch the Mixture Analysis Tool 43
- Step 2: Import a Population Database. 43
- Step 3: Create a New Mixture Analysis Method 45
- Step 4: Segregate the Samples 51

Overview

This chapter describes how to select and launch the Mixture Analysis tool from the Tools menu, select a population database, select and segregate samples, and define mixture analysis methods. You will create a new mixture analysis method during this process.

About Sample Segregation

Once you complete the preliminary preparation for Mixture Analysis described in Chapter 3, "Prepare for Mixture Analysis," you can perform sample segregation. You will create a new mixture analysis method in which you will define the peak-height ratio settings that are applied to different peak height ranges and the Mixture Interpretation Threshold. You can then proceed with sample segregation using the mixture analysis method you have defined.

Note: Applied Biosystems recommends creating a set of mixture analysis methods that correspond to the interpretation criteria developed by your laboratory based on internal validation studies. Different mixture analysis parameters may be appropriate for data generated using different AmpFℓSTR® kits, instrument platforms, or for particular sample types.

In This Chapter

In this chapter, you will learn to:

- Launch the Mixture Analysis Tool.
- Import a population database.
- Create a new mixture analysis method.
- Segregate the samples into one of the three classification groups: 1 contributor (single source), 2 contributors, or 3 or more contributors.

Step 1: Launch the Mixture Analysis Tool

1. Unless already open, launch the GeneMapper® *ID-X* Software Version 1.1 (v1.1):

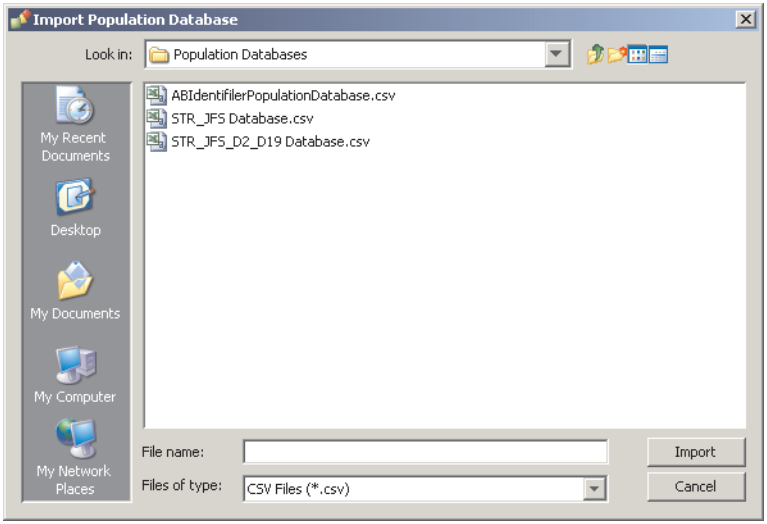
   Navigate to <*drive*>:\AppliedBiosystems\GeneMapper ID-X\Example Data\Mixture Analysis and select **MA_SGM**.

2. Select **Tools** ▸ **Mixture Analysis Manager** from the toolbar to open the Mixture Analysis Manager.

Step 2: Import a Population Database

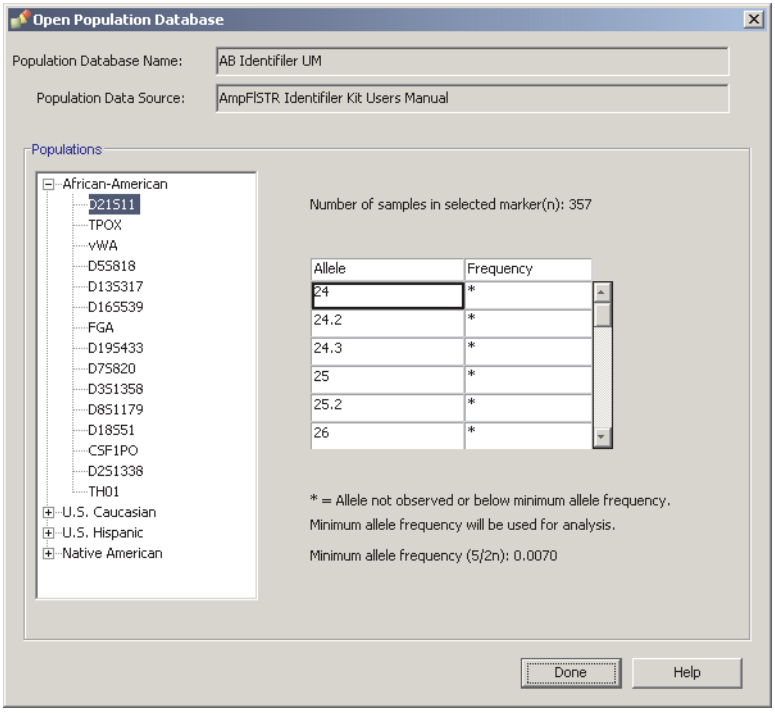
1. In the Mixture Analysis Manager, select the **Population Databases** tab and click **Import**.

2. Navigate, if necessary, to <*drive*>:\AppliedBiosystems\GeneMapperID-X\Mixture Analysis\Population Databases and select **ABIdentifilerPopulationDatabase.csv**, then click **Import**.



3. Select **Open** to view the AB Identifiler Population Database. This database is provided as a default with the GeneMapper® *ID-X* Software v1.1.

   The Open Population Database dialog box allows you to view the number of samples comprising each database, the allele frequency data, and the minimum allele frequency used for each database.



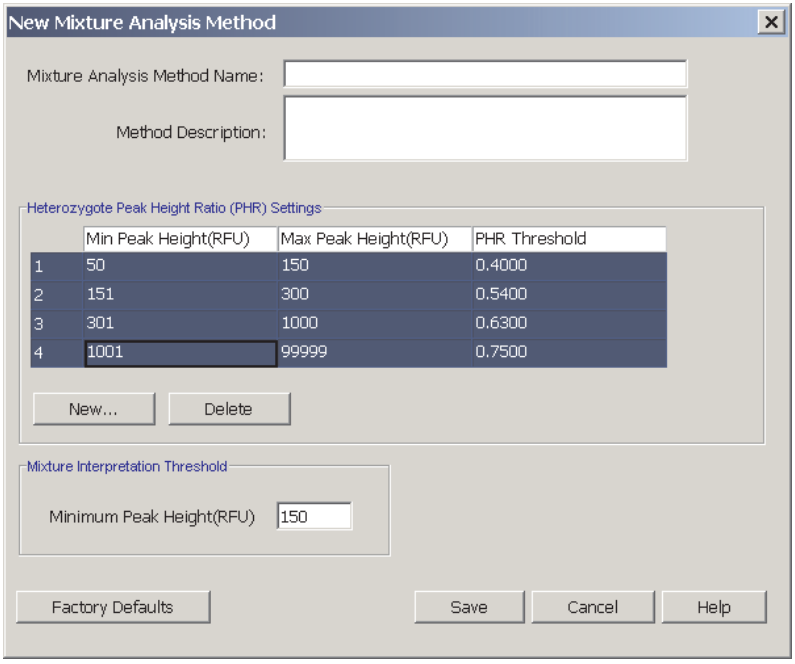
Step 3: Create a New Mixture Analysis Method

Create a Mixture Analysis Method using the Mixture Analysis Manager

1. In the Mixture Analysis Manager, select the **Mixture Analysis Methods** tab, then click **New** to open the New Mixture Analysis Method dialog box.
2. Review the existing parameters:
   - Mixture Analysis Method Name

     Note: The following symbols are not allowed in mixture analysis method file names: \\ / : * ? < > \ | ".
   - (Optional) Method Description
   - Heterozygote PHR Settings
   - Mixture Interpretation Threshold

IMPORTANT! The default entries in the Heterozygote Peak Height Ratio (PHR) Settings table below are for demonstration purposes only. Use an internal validation study to determine the appropriate settings to use for your interpretation, then use the following procedure to create new settings for your analysis. To change existing PHR settings, refer to *Editing Heterozygote Peak Height Ratio Settings* in the *GeneMapper® ID-X Software Help*.



3. In the Mixture Analysis Method Name: field of the New Mixture Analysis Method dialog box shown above, enter the name **MA Test Method**.

4. In the Mixture Interpretation Threshold - Minimum Peak Height (RFU) field, enter **150**.

5. In the Heterozygote Peak Height Ratio (PHR) Settings area of the dialog box, highlight all four rows as shown, then click **Delete** to remove all of the default settings.

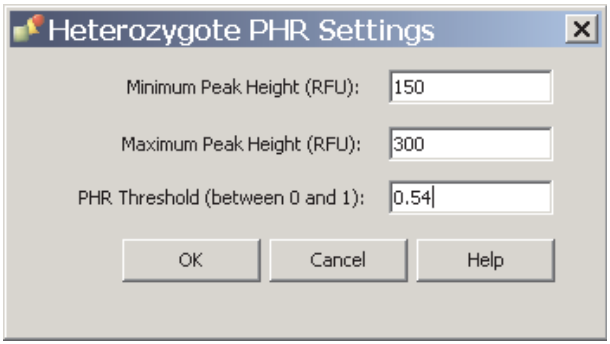
6. Click **New** to launch the Heterozygote PHR Settings dialog box, then enter the following:

   a. In the Minimum Peak Height (RFU) field, enter **150**.

   b. In the Maximum Peak Height (RFU) field, enter **300**.

   c. In the PHR Threshold (between 0 and 1) field, enter **0.54**.

   as shown in the next dialog box.



7. Click **OK** to save your changes and return to the New Mixture Analysis Method dialog box.

8. Click **New** to create a second PHR threshold and perform the following:

   a. Verify the Minimum Peak Height value is 301.

   Note: The default value for a new Minimum Peak Height entry in the Heterozygote PHR settings table = the Maximum Peak Height value for the previous row in the table + 1.

   b. In the Maximum Peak Height (RFU) field, enter **1000**.

   c. In the PHR Threshold (between 0 and 1) field, enter **0.60**.

9. Click **OK** to save your changes and return to the New Mixture Analysis Method dialog box.

10. Click **New** to create a third PHR threshold and perform the following:

    a. Verify the Minimum Peak Height value is 1001.

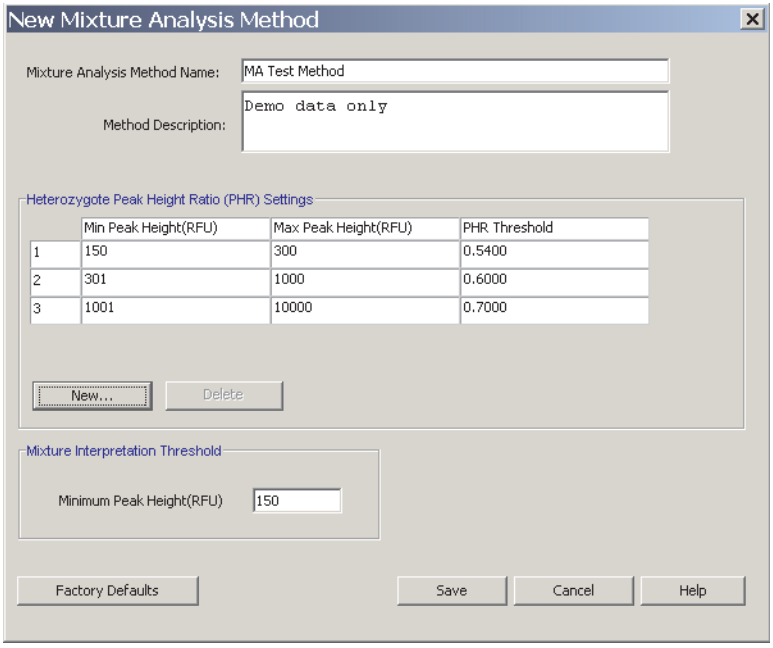
    Note: The default value for a new Minimum Peak Height entry in the Heterozygote PHR settings table = the Maximum Peak Height value for the previous row in the table + 1.

    b. In the Maximum Peak Height (RFU) field, enter **10000**.

c. In the PHR Threshold (between 0 and 1) field, enter **0.70**.

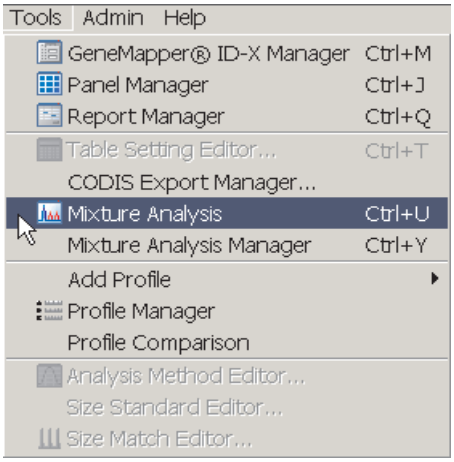
11. Click **OK** to save your changes and return to the New Mixture Analysis Method dialog box.

    The final mixture analysis test method displays the following settings.

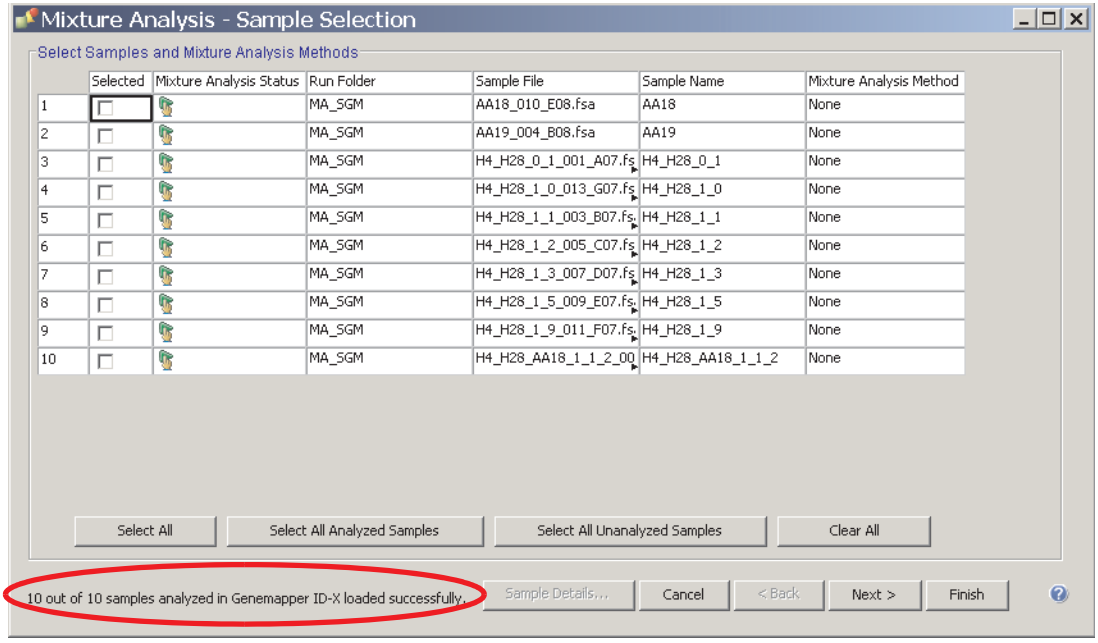


Apply a Mixture Analysis Method to Samples

1. Select **Tools** ▸ **Mixture Analysis** from the toolbar or press **Ctrl+ U.**



The Mixture Analysis - Sample Selection dialog box appears.



Note: Note that all 10 samples loaded successfully.

2. Click **Select All** to select all of the samples (check marks appear in each checkbox).

3. In the Mixture Analysis Method column, click the first sample, click **New**, then select **MA Test Method** from the drop-down menu.

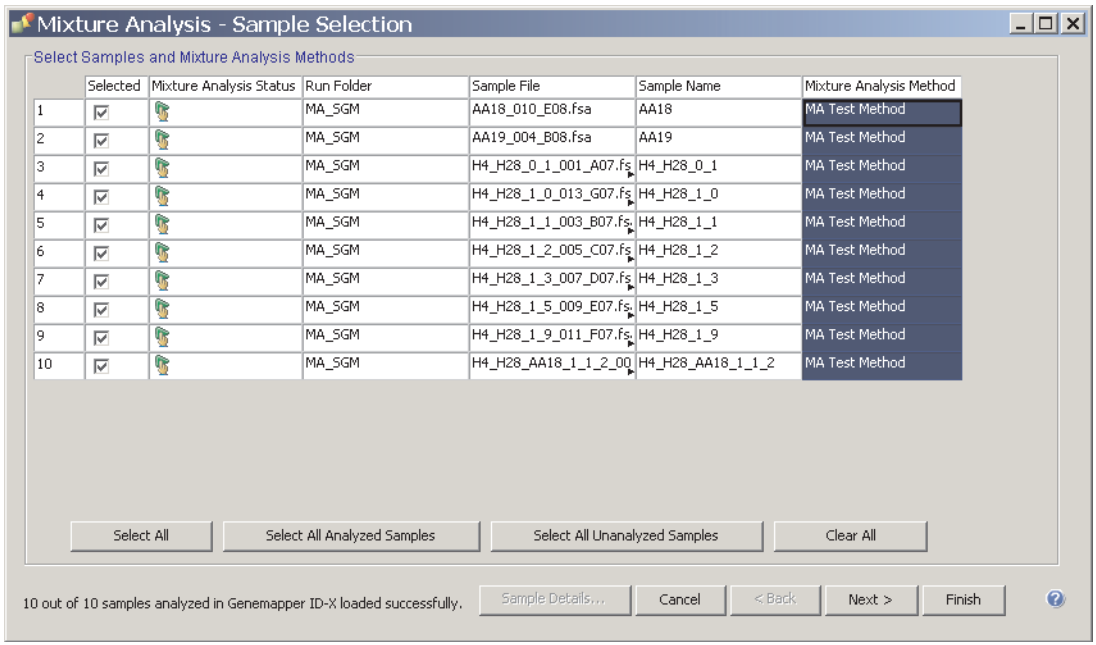
4. Click on the Mixture Analysis Method column header and press **Ctrl-D** to apply the MA Test Method mixture analysis method to all samples.

Create an MA Method using the Column Drop-Down Method (Alternate Method)

You can also create new mixture analysis methods by selecting **New** from the Mixture Analysis Method column drop-down menu, then following the directions in "Create a Mixture Analysis Method using the Mixture Analysis Manager" on page 45.

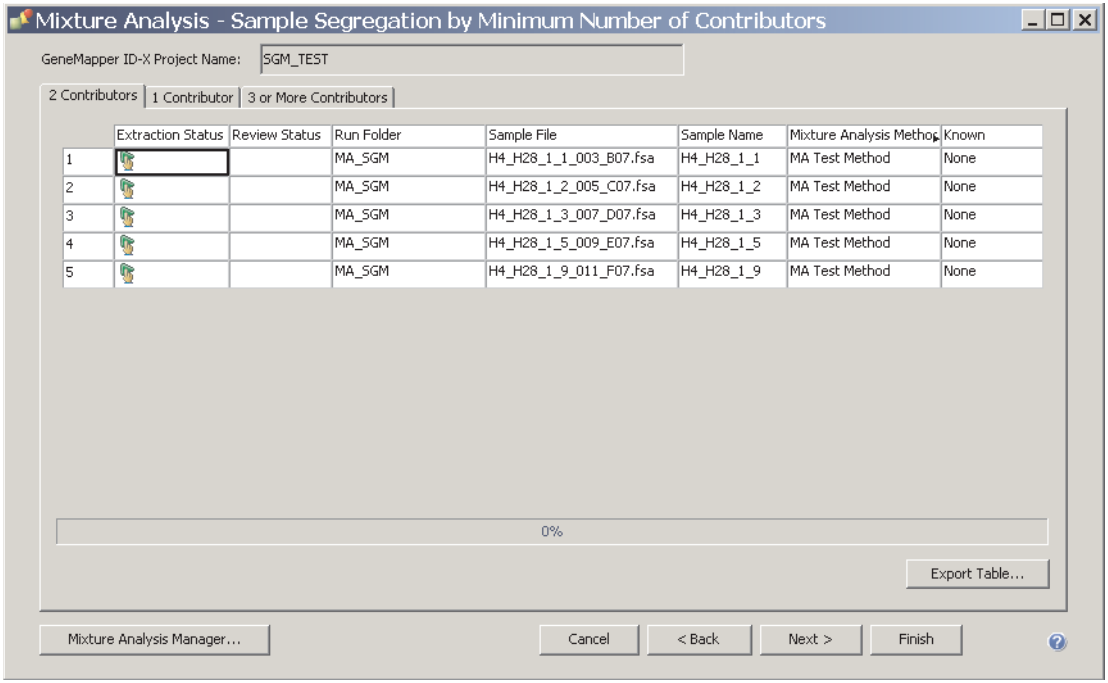
Step 4: Segregate the Samples

After you've applied the MA Test Method mixture analysis method to all samples, the Mixture Analysis - Sample Selection dialog box should appear as shown below.

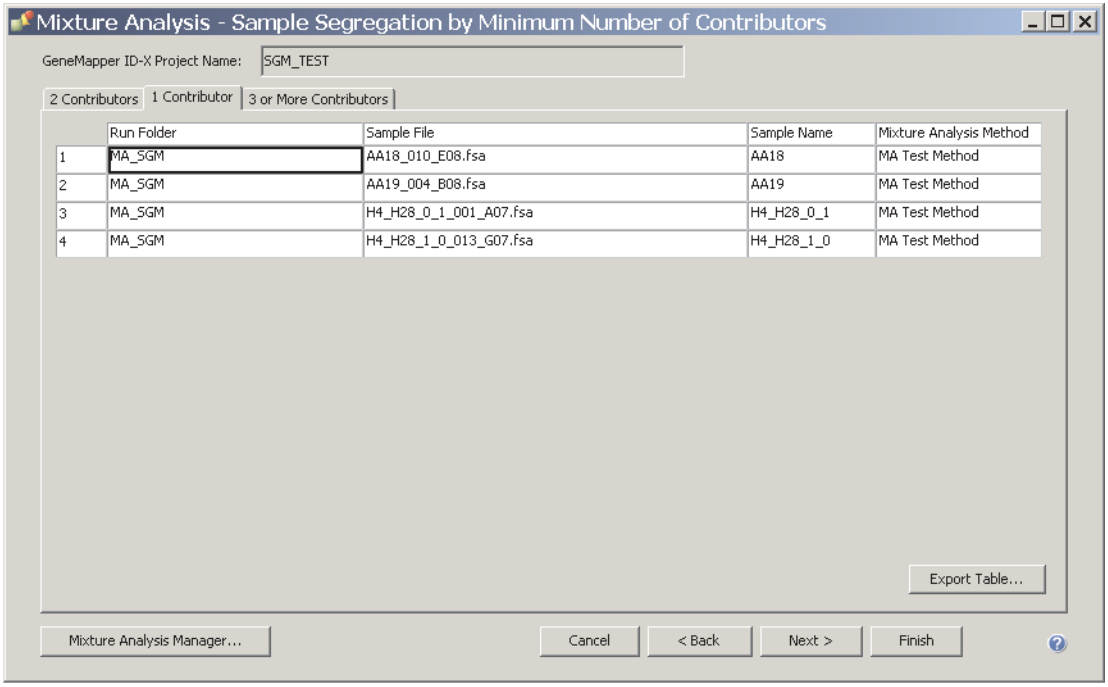

Mixture Analysis - Sample Selection
Select Samples and Mixture Analysis Methods
Selected
Mixture Analysis Status
Run Folder
Sample File
Sample Name
Mixture Analysis Method
1 MA_SGM AA18_010_E08.fsa AA18 MA Test Method
2 MA_SGM AA19_004_B08.fsa AA19 MA Test Method
3 MA_SGM H4_H28_0_1_001_A07.fs H4_H28_0_1 MA Test Method
4 MA_SGM H4_H28_1_0_013_G07.fs H4_H28_1_0 MA Test Method
5 MA_SGM H4_H28_1_1_003_B07.fs H4_H28_1_1 MA Test Method
6 MA_SGM H4_H28_1_2_005_C07.fs H4_H28_1_2 MA Test Method
7 MA_SGM H4_H28_1_3_007_D07.fs H4_H28_1_3 MA Test Method
8 MA_SGM H4_H28_1_5_009_E07.fs H4_H28_1_5 MA Test Method
9 MA_SGM H4_H28_1_9_011_F07.fs H4_H28_1_9 MA Test Method
10 MA_SGM H4_H28_AA18_1_1_2_00 H4_H28_AA18_1_1_2 MA Test Method
Select All
Select All Analyzed Samples
Select All Unanalyzed Samples
Clear All
10 out of 10 samples analyzed in Genemapper ID-X loaded successfully.
Sample Details...
Cancel
< Back
Next >
Finish

1. Click **Next>**, then click each of the contributor tabs to review the results of the sample segregation process as follows.

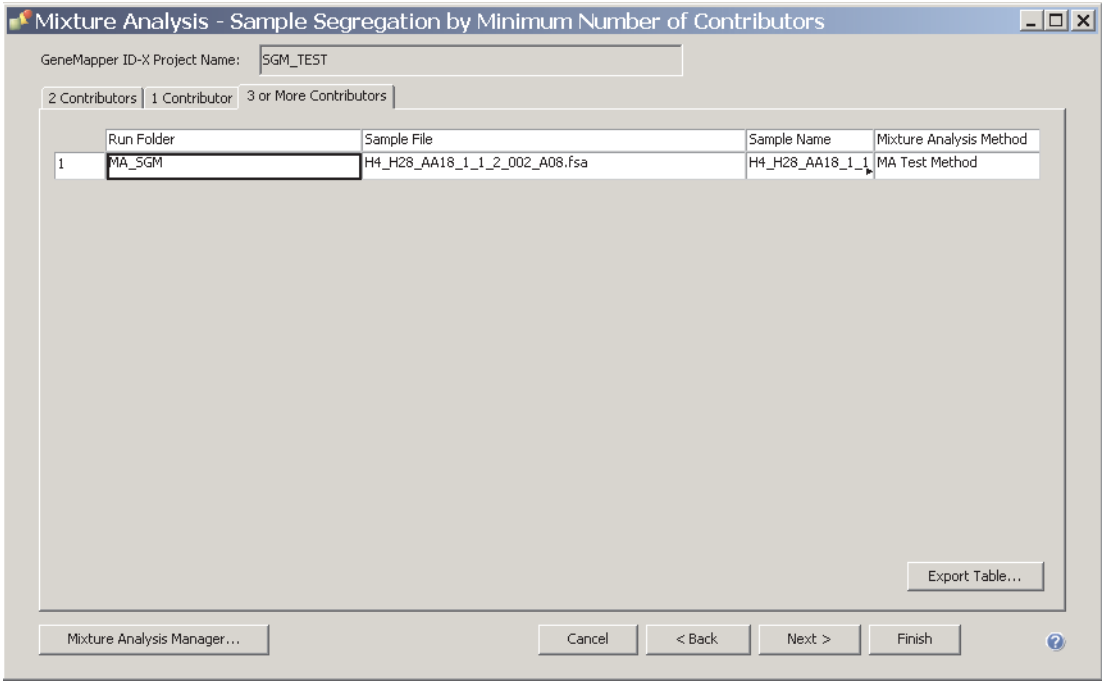
2. Review the **2 Contributors** tab results: 5 samples were segregated as mixtures with a minimum of 2 contributors.



3. Review the **1 Contributor** tab: 4 samples were determined to originate from a single contributor.



4. Review the **3 or More Contributors** tab: 1 sample was identified by the software as containing 3 or more contributors.

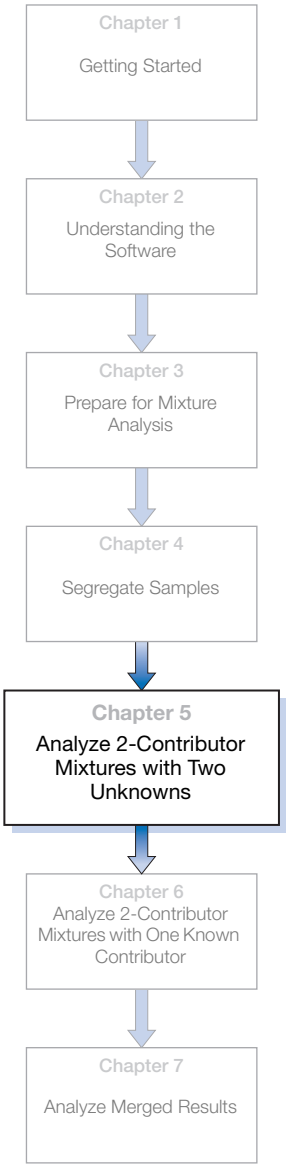


IMPORTANT! Saved sample segregation results are overwritten when you reanalyze a sample.

5. Go back to the **2 Contributors** tab and continue to Chapter 5 to extract 2-contributor mixtures.

Refer to the *GeneMapper® ID-X Software Help* for additional information on segregating samples.

5 Analyze 2-Contributor Mixtures with Two Unknowns



This chapter covers:

- Overview 56
- Step 1: Extract Individual Genotypes 57
- Step 2: Review the Results of the Extraction 58
- Step 3: Determine Genotype Combinations to Include in Statistical Calculations 63
- Step 4: Calculate Random Match Probabilities and Combined Probability of Inclusion/Exclusion 71
- Step 5: Review the Results of Random Match Probability and Combined Probability of Inclusion/Exclusion Calculations. . 72
- Step 6: Calculate and Review Likelihood Ratio Results 74

Overview

About Analysis of 2-Contributor Samples Where Both Contributors are Unknown

When analyzing 2-contributor mixtures where both contributors are unknown, the software extracts the possible genotype combinations for each contributor and assesses each combination according to the peak height ratio thresholds set in the Mixture Analysis Method and the residual threshold specified by the software.

The software:

- Generates all possible genotype combinations at each marker.
- Calculates the residual values and peak height ratios for all combinations.
- Computes an average minor contributor mixture proportion (Mx) from 3-peak and 4-peak data across a profile, which serves as the basis for comparison of all genotype combinations generated.
- Automatically selects combinations that meet the criteria for inclusion in the statistical calculations.

You can review the results of the extraction and select genotype combinations for statistical analysis.

For a discussion of mixture analysis methods, refer to Gill *et al*., 1998 (see Bibliography). For additional information on 2-contributor samples, refer to the *GeneMapper® ID-X Software Help*.

In This Chapter

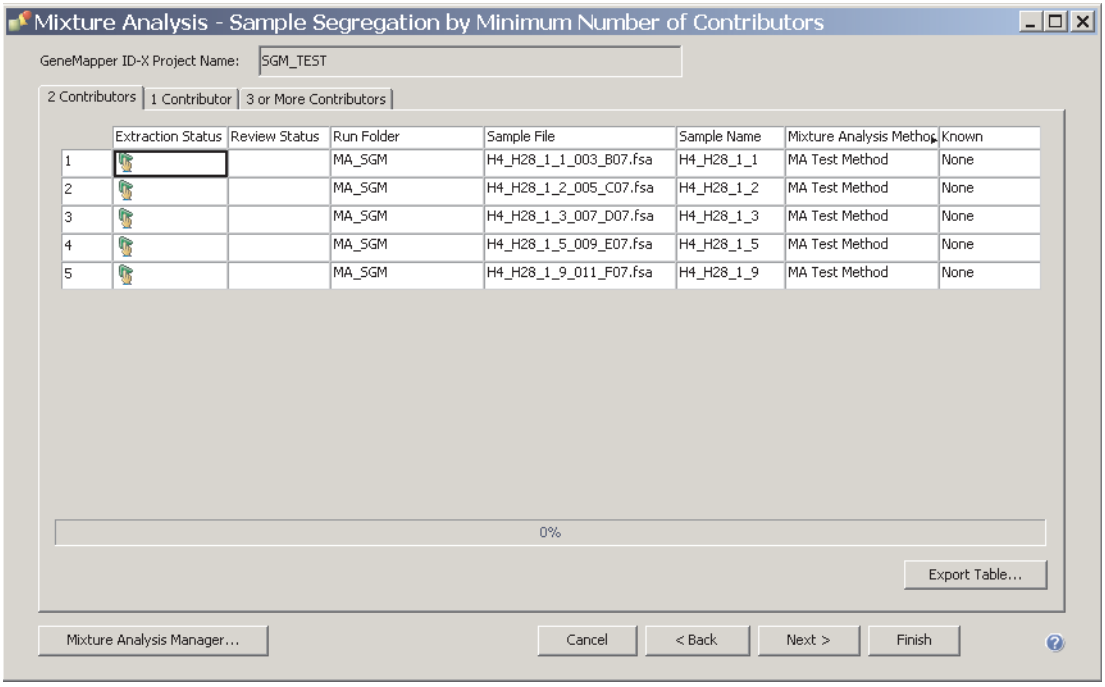
In this chapter, you will learn to:

- Extract individual genotypes from possible contributors of a two-person mixture where both contributors are unknown.
- Use the GeneMapper® *ID-X* Mixture Analysis Quality Value system to review the results of the extraction.
- Determine which genotype combinations to include in the statistical calculations.
- Calculate Random Match Probabilities (RMP) for each individual contributor to the mixture.
- Calculate the Combined Probability of Inclusion/Exclusion (CPI/CPE) for the mixed sample.

- Review the results of the RMP and CPI/CPE calculations.
- Calculate and review Likelihood Ratios (LR) results.

Step 1: Extract Individual Genotypes

1. Select the **2 Contributors** tab in the Mixture Analysis Sample Segregation dialog box. Five samples are displayed as shown below.



The dialog box buttons specific to the 2-contributor mixture workflow are:

- **Export Table** - Exports the table of samples characterized as minimum 2-contributor mixtures
- **Next >** - Performs new extraction. Performs extraction of the mixture to identify the individual genotypes of the 2 contributors present in the mixture
- **<Back** - Returns to the previous dialog box

- **Finish** - Returns to the GeneMapper ID-X Samples table

2. Click **Next >** to display the Mixture Analysis Results Viewer.

Step 2: Review the Results of the Extraction

About Mixture Analysis Results Viewer Tables

The Mixture Analysis Results Viewer contains two data tables to aid in the review of the extraction results.

Information Found in Tables

The data appearing automatically in each table are determined by the GeneMapper® *ID-X* Mixture Analysis Quality Value System . The system assists with review and interpretation of the data produced through the genotype extraction process.

The system assesses each possible genotype combination to determine whether the following thresholds are met:

- **The peak-height ratio (PHR) thresholds set in the Mixture Analysis Method.** The peak-height ratio settings were described in Chapter 3, "Prepare for Mixture Analysis," when the mixture analysis method was created.

  and

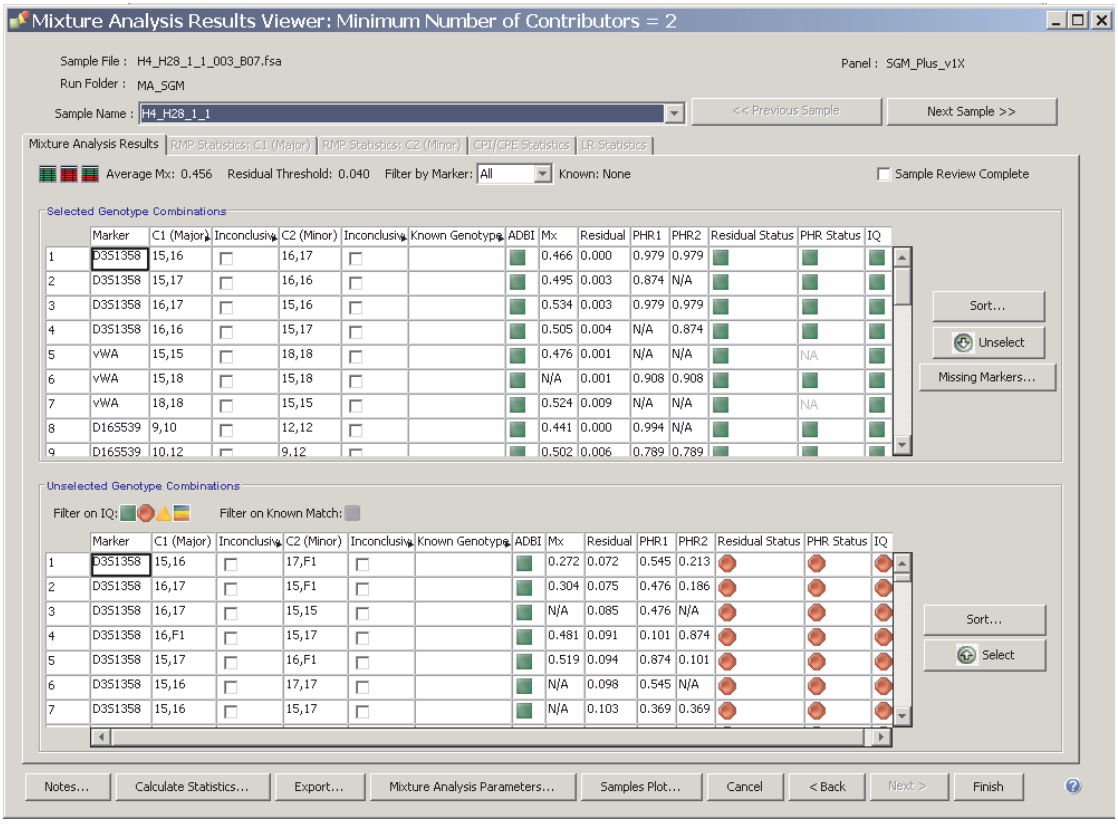
- **The residual threshold specified by the software.** The residual is a measure of how close the minor contributor proportion for a particular genotype combination is to the expected minor contributor proportion for a particular profile.

The combination of the above measures determines the inclusion quality (IQ) value assigned to the genotype combination.

The PQV results of these quality assessment tools are displayed using the same color coded flags as employed by the GeneMapper® *ID-X* Software during data analysis.

The color of the flag depends on software-specified thresholds or user-defined thresholds set in the mixture analysis method. Genotype combinations with a passing green ( ) IQ are automatically assigned to the Selected Genotype Combinations table for inclusion in statistical calculations.

The Mixture Analysis Results Viewer is shown in the following figure.


Mixture Analysis Results Viewer: Minimum Number of Contributors = 2
Sample File : H4_H28_1_1_003_B07.fsa
Panel : SGM_Plus_v1X
Run Folder : MA_SGM
Sample Name : H4_H28_1_1
<< Previous Sample
Next Sample >>
Mixture Analysis Results
RMP Statistics: C1 (Major)
RMP Statistics: C2 (Minor)
CPI/CPE Statistics
LR Statistics
Average Mx: 0.456
Residual Threshold: 0.040
Filter by Marker: All
Known: None
Sample Review Complete
Selected Genotype Combinations
Marker | C1 (Major) | Inconclusive | C2 (Minor) | Inconclusive | Known Genotype | ADBI | Mx | Residual | PHR1 | PHR2 | Residual Status | PHR Status | IQ
1 D3S1358 15,16 16,17 0.466 0.000 0.979 0.979
2 D3S1358 15,17 16,16 0.495 0.003 0.874 N/A
3 D3S1358 16,17 15,16 0.534 0.003 0.979 0.979
4 D3S1358 16,16 15,17 0.505 0.004 N/A 0.874
5 vWA 15,15 18,18 0.476 0.001 N/A N/A NA
6 vWA 15,18 15,18 N/A 0.001 0.908 0.908
7 vWA 18,18 15,15 0.524 0.009 N/A N/A NA
8 D16S539 9,10 12,12 0.441 0.000 0.994 N/A
9 D16S539 10,12 9,12 0.502 0.006 0.789 0.789
Sort...
Unselect
Missing Markers...
Unselected Genotype Combinations
Filter on IQ:
Filter on Known Match:
Marker | C1 (Major) | Inconclusive | C2 (Minor) | Inconclusive | Known Genotype | ADBI | Mx | Residual | PHR1 | PHR2 | Residual Status | PHR Status | IQ
1 D3S1358 15,16 17,F1 0.272 0.072 0.545 0.213
2 D3S1358 16,17 15,F1 0.304 0.075 0.476 0.186
3 D3S1358 16,17 15,15 N/A 0.085 0.476 N/A
4 D3S1358 16,F1 15,17 0.481 0.091 0.101 0.874
5 D3S1358 15,17 16,F1 0.519 0.094 0.874 0.101
6 D3S1358 15,16 17,17 N/A 0.098 0.545 N/A
7 D3S1358 15,16 15,17 N/A 0.103 0.369 0.369
Sort...
Select
Notes...
Calculate Statistics...
Export...
Mixture Analysis Parameters...
Samples Plot...
Cancel
< Back
Next >
Finish

Selected Genotype Combinations Table

The Selected Genotype Combinations table shows the list of possible genotype combinations, deducible from the mixture, that passed the criteria for selection (IQ status) and were therefore automatically assigned to this table for inclusion in the statistical calculations.

To exclude genotype combinations from the statistical calculations, highlight the genotype combination(s) and click the **Unselect** button to the right of the table.

Unselected Genotype Combinations Table

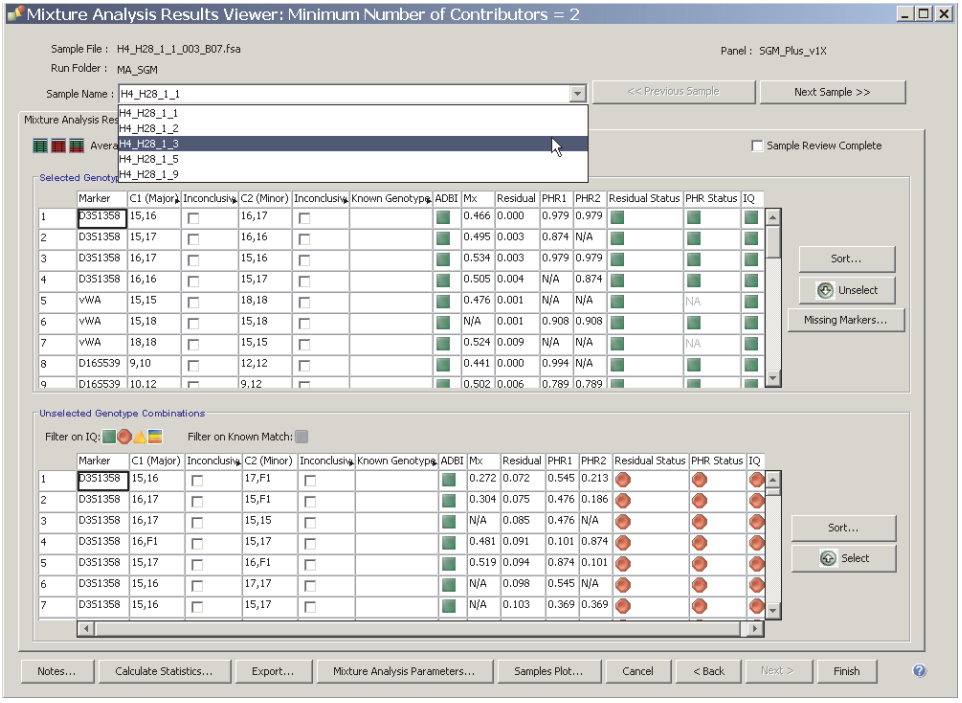
The Unselected Genotype Combinations table shows the list of possible genotype combinations, deducible from the mixture, which failed to pass the criteria for automatic inclusion in the Selected Genotype Combinations table.

To include these genotype combinations in statistical calculations, highlight the required genotype combination and click the **Select** button to the right of the table. This action moves the combinations from the Unselected Genotypes Combinations table to the Selected Genotypes Combinations table.

Review Extraction Results

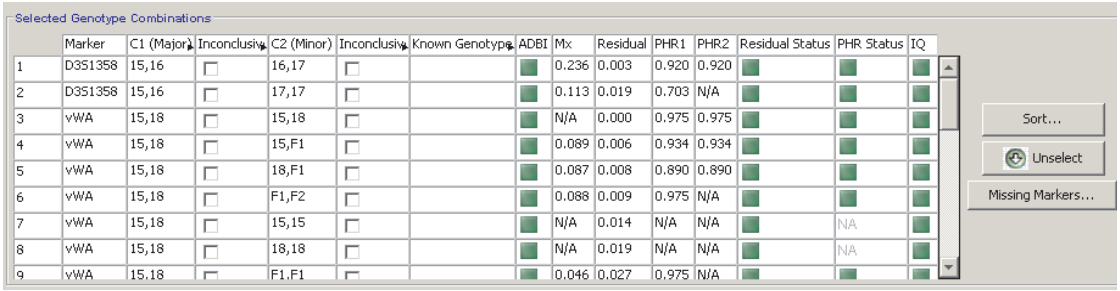
To review the results of the extraction:

1. Select sample H4_H28_1_3 from the drop down menu.


Mixture Analysis Results Viewer: Minimum Number of Contributors = 2
Sample File : H4_H28_1_1_003_B07.fsa
Panel : SGM_Plus_v1X
Run Folder : MA_SGM
Sample Name : H4_H28_1_1
<< Previous Sample
Next Sample >>
H4_H28_1_1
H4_H28_1_2
H4_H28_1_3
H4_H28_1_5
H4_H28_1_9
Sample Review Complete
Selected Genotyp
Marker C1 (Major) Inconclusiv C2 (Minor) Inconclusiv Known Genotype ADBI Mx Residual PHR1 PHR2 Residual Status PHR Status IQ
1 D3S1358 15,16 16,17 0.466 0.000 0.979 0.979
2 D3S1358 15,17 16,16 0.495 0.003 0.874 N/A
3 D3S1358 16,17 15,16 0.534 0.003 0.979 0.979
4 D3S1358 16,16 15,17 0.505 0.004 N/A 0.874
5 vWA 15,15 18,18 0.476 0.001 N/A N/A NA
6 vWA 15,18 15,18 N/A 0.001 0.908 0.908
7 vWA 18,18 15,15 0.524 0.009 N/A N/A NA
8 D16S539 9,10 12,12 0.441 0.000 0.994 N/A
9 D16S539 10,12 9,12 0.502 0.006 0.789 0.789
Sort...
Unselect
Missing Markers...
Unselected Genotype Combinations
Filter on IQ:
Filter on Known Match:
Marker C1 (Major) Inconclusiv C2 (Minor) Inconclusiv Known Genotype ADBI Mx Residual PHR1 PHR2 Residual Status PHR Status IQ
1 D3S1358 15,16 17,F1 0.272 0.072 0.545 0.213
2 D3S1358 16,17 15,F1 0.304 0.075 0.476 0.186
3 D3S1358 16,17 15,15 N/A 0.085 0.476 N/A
4 D3S1358 16,F1 15,17 0.481 0.091 0.101 0.874
5 D3S1358 15,17 16,F1 0.519 0.094 0.874 0.101
6 D3S1358 15,16 17,17 N/A 0.098 0.545 N/A
7 D3S1358 15,16 15,17 N/A 0.103 0.369 0.369
Sort...
Select
Notes...
Calculate Statistics...
Export...
Mixture Analysis Parameters...
Samples Plot...
Cancel
< Back
Next >
Finish

2. Review the data automatically assigned to the Selected Genotype Combinations table for sample H4_H28_1_3.

Note: All selected genotype combinations show a green ( ) IQ flag.

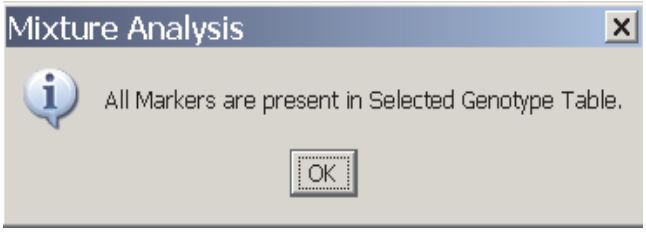


Additional columns display the following:

- The Mixture Proportion (Mx) value for each genotype combination. Where calculation of the Mx value is not possible, N/A is displayed.
- The residual value for each genotype combination.
- The peak height ratio for the C1(Major) contributor (PHR1) and for the C2 (Minor) contributor (PHR2). Where calculation of the peak height ratio is not possible (for a homozygous genotype) N/A is displayed.
- The Residual Status, which displays a green ( ) IQ value if the calculated residual is below the residual threshold.
- The PHR Status, which displays a green ( ) IQ value if all calculated peak height ratios exceed the thresholds set in the Mixture Analysis Method.

Note: The ADBI (Above Detection Below Interpretation) column in the table displays a yellow ( ) flag when any allele peak height within a marker genotype combination falls above the minimum peak amplitude threshold set in the GeneMapper® *ID-X* Software, and below the Mixture Interpretation Threshold (MIT) set in the mixture analysis method.

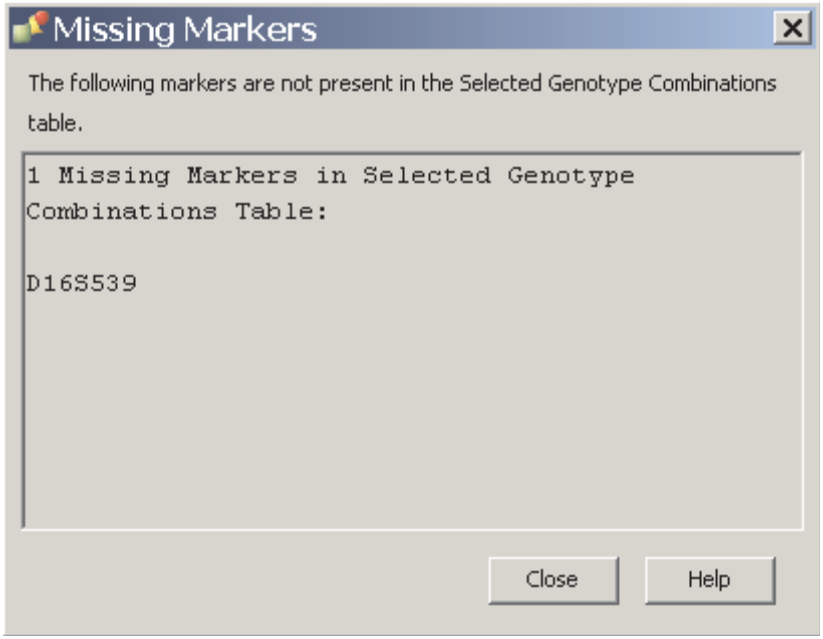
3. Select the **Missing Markers...** button. The following message is displayed:



This message indicates that at least one genotype combination was selected for each of the markers detected for the sample.

4. Click **OK**.

For instances where a detected marker does not have any combinations in the Selected Genotype Combinations table, a dialog box similar to the one below is displayed. Click **Close**.


Missing Markers
The following markers are not present in the Selected Genotype Combinations table.
1 Missing Markers in Selected Genotype Combinations Table:
D16S539
Close
Help

5. Review the data contained in the Unselected Genotype Combinations table. All possible genotype combinations show either a yellow or a red IQ flag. These combinations were not automatically selected by the software because they failed to meet one or more criteria.

Note: You can also filter both tables according to IQ status by clicking a **Filter on IQ** icon

.

Step 3: Determine Genotype Combinations to Include in Statistical Calculations

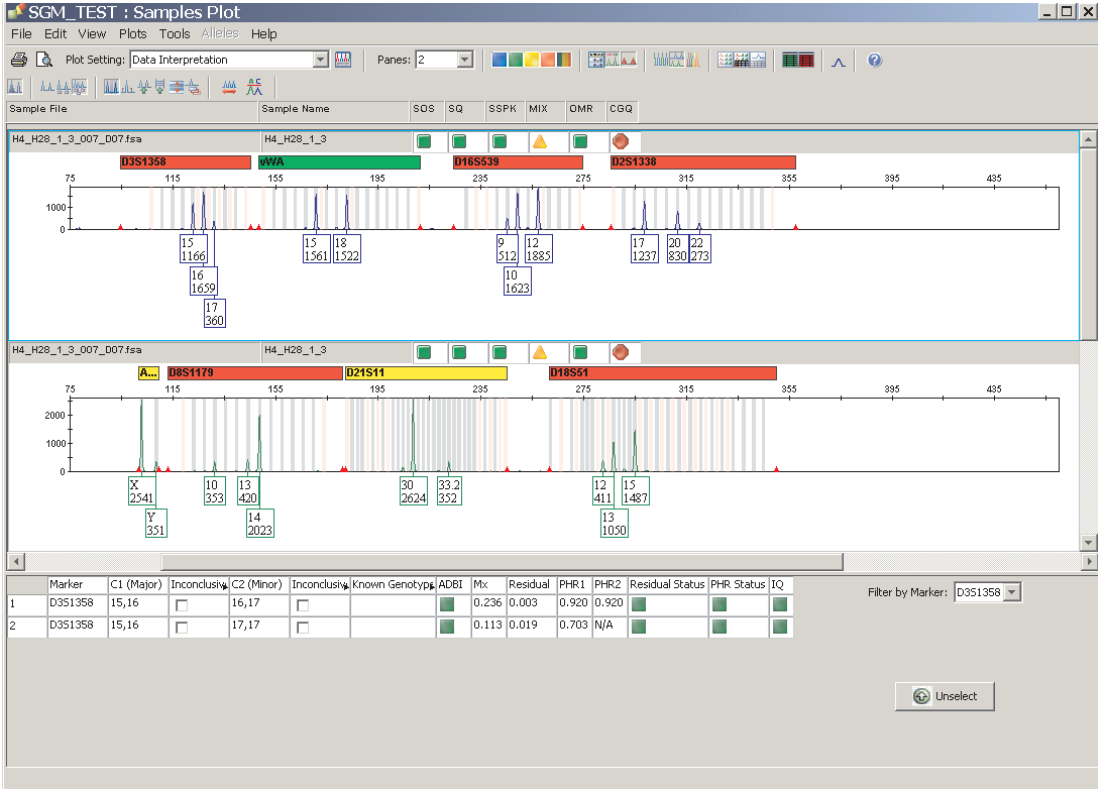
Review First Sample

1. Select sample H4_H28_1_3 from the drop down menu..
2. Click the **Samples Plot…** button, then **OK** in the popup dialog box that explains the editing functions to view the electropherogram for mixture sample H4_H28_1_3. The editing functions are disabled for the Mixture Analysis Tool Sample Plot, however, other functions such as the Plot Setting Editor, tables, and viewing functions are enabled.
3. Select the following icon on the Samples Plot to display the Mixture Analysis Selected Genotype Combinations table below the Sample Plot.

4. Select **2** in the **Panes** drop-down list to simultaneously view the electropherogram information and the table.

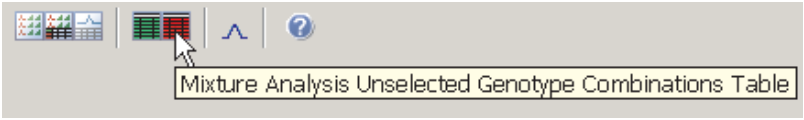
5. Select **Filter by Marker: D3S1358** to the right of the Selected Genotype Combinations table to filter the results as shown.

Note: You can also highlight the genotype combinations corresponding to a particular marker without filtering the table by clicking on the marker header bar for that marker.



6. Review the selected genotype combinations for the D3S1358 marker to assess whether the software successfully evaluated the 2-contributor mixture. Use the marker filter or marker header bar selection to review the data for other markers.

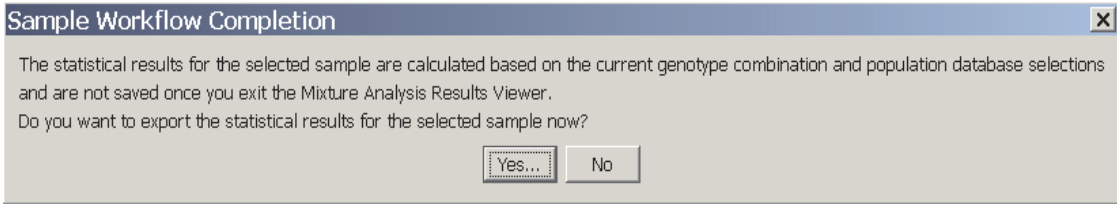
7. Select the icon indicated below to display the Mixture Analysis Unselected Genotype Combinations table and view the combinations that the software did not automatically select.



For each marker, verify whether you agree with the combinations that were NOT selected. Use the **Select** button to manually add genotype combinations to the Selected Genotype Combinations table.

8. Click the **X** icon in the upper right-hand corner to exit the Samples Plot and return to the Mixture Analysis Results Viewer.

9. Check the **Sample Review Complete** box to indicate that this sample is ready for statistical analysis. The Sample Workflow Completion dialog box is displayed.


Sample Workflow Completion
The statistical results for the selected sample are calculated based on the current genotype combination and population database selections and are not saved once you exit the Mixture Analysis Results Viewer.
Do you want to export the statistical results for the selected sample now?
Yes...
No

10. Click **No.**

Note: You will calculate statistics in "Step 4: Calculate Random Match Probabilities and Combined Probability of Inclusion/Exclusion."

Review Second Sample

1. Select **Next Sample >>** to review sample H4_H28_1_5.

2. Click the **Samples Plot** button, then click **OK** in the popup dialog box that explains the editing functions.

3. Review the Selected Genotype Combinations table.

4. Select **Filter by Marker: D3S1358.**

   The following combinations are selected for D3S1358.

Selected Genotype Combinations

	Marker	C1 (Major)	Inconclusiv	C2 (Minor)	Inconclusiv	Known Genotype	ADBI	Mx	Residual	PHR1	PHR2	Residual Status	PHR Status	IQ
1	D3S1358	15,16	☐	17,F1	☐		■	0.151	0.001	0.994	0.542	■	■	■
2	D3S1358	15,16	☐	17,17	☐		■	0.103	0.002	0.994	N/A	■	■	■
3	D3S1358	15,16	☐	16,17	☐		■	0.187	0.004	0.809	0.809	■	■	■
4	D3S1358	15,16	☐	15,17	☐		■	0.187	0.004	0.817	0.817	■	■	■

Note: The combination in row 1 contains a minor contributor genotype of 17, F1. In this case, the software analyzes the possibility of allele dropout below the Mixture Interpretation threshold in order to include the combination.

5. From the Samples Plot, select row 1 in the Selected Genotype Combinations table.

6. Click **Unselect**.

   The combination in row 1 moves to the Unselected Genotype Combinations table and no longer appears in the Selected Genotype Combinations table. This removed combination will not be included in the statistical calculations for this sample.

Note: This combination is being removed for the purposes of this demonstration only.

7. Select the icon indicated below to display the Unselected Genotype Combinations table.

Select **Filter by Marker:** D3S1358 to display the results, as shown below.

	Marker	C1 (Major)	Inconclusiv	C2 (Minor)	Inconclusiv	Known Genotype	ADBI	Mx	Residual	PHR1	PHR2	Residual Status	PHR Status	IQ
1	D3S1358	15,16	☐	17,F1	☐		■	0.151	0.001	0.994	0.542	■	■	■
2	D3S1358	15,17	☐	16,16	☐		■	N/A	0.202	0.229	N/A	●	●	●
3	D3S1358	16,17	☐	15,15	☐		■	N/A	0.203	0.231	N/A	●	●	●
4	D3S1358	15,17	☐	16,F1	☐		■	0.476	0.236	0.229	0.125	●	●	●
5	D3S1358	16,17	☐	15,F1	☐		■	0.479	0.238	0.231	0.124	●	●	●
6	D3S1358	15,17	☐	15,16	☐		■	0.813	0.252	0.817	0.817	●	■	▲
7	D3S1358	16,17	☐	15,16	☐		■	0.813	0.254	0.809	0.809	●	■	▲
8	D3S1358	15,F1	☐	16,17	☐		■	0.521	0.268	0.124	0.231	●	●	●
9	D3S1358	16,F1	☐	15,17	☐		■	0.524	0.270	0.125	0.229	●	●	●
10	D3S1358	15,17	☐	16,17	☐		■	N/A	0.300	0.115	0.115	●	●	●
11	D3S1358	16,17	☐	15,17	☐		■	N/A	0.302	0.115	0.115	●	●	●
12	D3S1358	15,15	☐	16,17	☐		■	N/A	0.312	N/A	0.231	●	●	●
13	D3S1358	16,16	☐	15,17	☐		■	N/A	0.316	N/A	0.229	●	●	●
14	D3S1358	17,F1	☐	15,16	☐		■	0.849	0.505	0.542	0.994	●	■	▲
15	D3S1358	17,17	☐	15,16	☐		■	0.897	0.860	N/A	0.994	●	■	▲

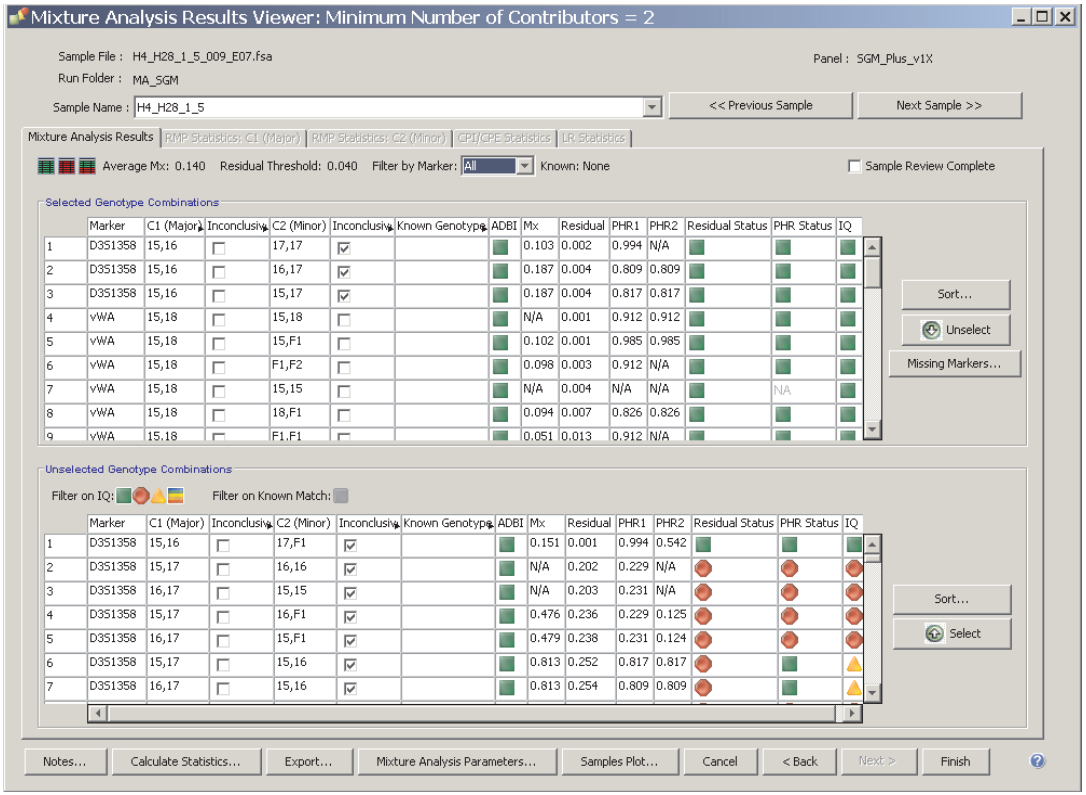
Note: The combination that you removed from the Selected Genotype Combinations table now appears in row 1 of the Unselected Genotype Combinations table.

8. Exit the Samples Plot by clicking the **X** in the upper right corner, then select **Filter by Marker: All** in the Mixture Analysis Results Viewer.

Select Genotypes for Second Sample

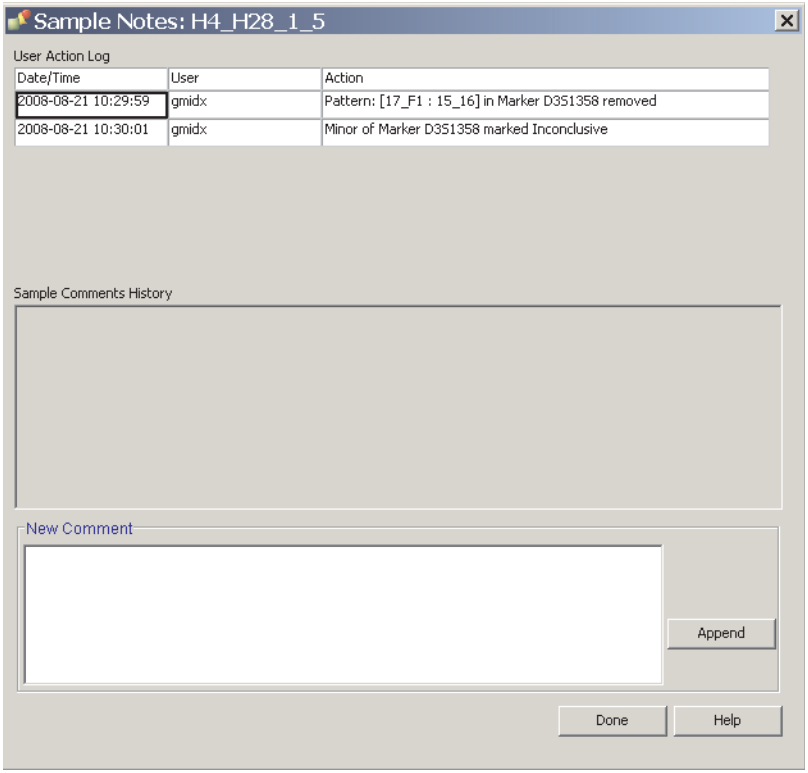
Assume that the D3S1358 marker cannot be interpreted with confidence for the minor contributor for sample H4_H28_1_5.

1. Omit the marker from the statistical calculations by checking the **Inconclusive (Minor) box** relating to any one of the D3S1358 possible genotype combinations in the Selected Genotype Combinations table.


Mixture Analysis Results Viewer: Minimum Number of Contributors = 2
Sample File : H4_H28_1_5_009_E07.fsa
Panel : SGM_Plus_v1X
Run Folder : MA_SGM
Sample Name : H4_H28_1_5
<< Previous Sample
Next Sample >>
Mixture Analysis Results
RMP Statistics: C1 (Major)
RMP Statistics: C2 (Minor)
CPI/CPE Statistics
LR Statistics
Average Mx: 0.140
Residual Threshold: 0.040
Filter by Marker: All
Known: None
Sample Review Complete
Selected Genotype Combinations
Marker | C1 (Major) | Inconclusive | C2 (Minor) | Inconclusive | Known Genotype | ADBI | Mx | Residual | PHR1 | PHR2 | Residual Status | PHR Status | IQ
1 D3S1358 15,16 17,17 0.103 0.002 0.994 N/A
2 D3S1358 15,16 16,17 0.187 0.004 0.809 0.809
3 D3S1358 15,16 15,17 0.187 0.004 0.817 0.817
4 vWA 15,18 15,18 N/A 0.001 0.912 0.912
5 vWA 15,18 15,F1 0.102 0.001 0.985 0.985
6 vWA 15,18 F1,F2 0.098 0.003 0.912 N/A
7 vWA 15,18 15,15 N/A 0.004 N/A N/A NA
8 vWA 15,18 18,F1 0.094 0.007 0.826 0.826
9 vWA 15,18 F1,F1 0.051 0.013 0.912 N/A
Sort...
Unselect
Missing Markers...
Unselected Genotype Combinations
Filter on IQ:
Filter on Known Match:
Marker | C1 (Major) | Inconclusive | C2 (Minor) | Inconclusive | Known Genotype | ADBI | Mx | Residual | PHR1 | PHR2 | Residual Status | PHR Status | IQ
1 D3S1358 15,16 17,F1 0.151 0.001 0.994 0.542
2 D3S1358 15,17 16,16 N/A 0.202 0.229 N/A
3 D3S1358 16,17 15,15 N/A 0.203 0.231 N/A
4 D3S1358 15,17 16,F1 0.476 0.236 0.229 0.125
5 D3S1358 16,17 15,F1 0.479 0.238 0.231 0.124
6 D3S1358 15,17 15,16 0.813 0.252 0.817 0.817
7 D3S1358 16,17 15,16 0.813 0.254 0.809 0.809
Sort...
Select
Notes...
Calculate Statistics...
Export...
Mixture Analysis Parameters...
Samples Plot...
Cancel
< Back
Next >
Finish

Note: When you assign one possible genotype combination as inconclusive for any marker, the software automatically checks the Inconclusive box for all other possible genotype combinations at that marker in both the Unselected and Selected Genotype Combinations tables.

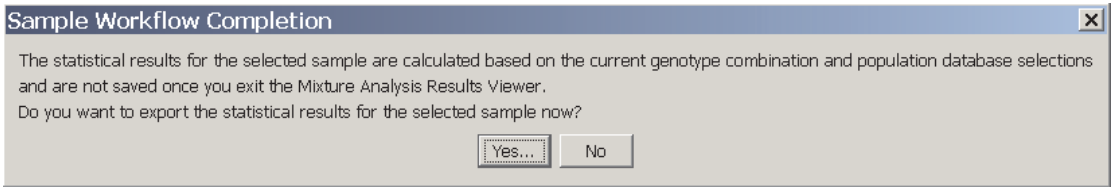
2. In the Mixture Analysis Results Viewer, click the **Notes…** button. The Sample Notes dialog box is displayed.



The dialog box shows that the software recorded the removal of pattern [17_F1:15_16] from the Selected Genotype Combinations table for marker D3S1358 and that D3S1358 is marked inconclusive for the minor contributor.

3. Click **Done** to exit the dialog box.

4. Check the **Sample Review Complete** checkbox to signify that review of the sample and selection of genotypes are finished and the sample is ready for statistical analysis. The Sample Workflow Completion dialog box is displayed.


Sample Workflow Completion
The statistical results for the selected sample are calculated based on the current genotype combination and population database selections and are not saved once you exit the Mixture Analysis Results Viewer.
Do you want to export the statistical results for the selected sample now?
Yes...
No

5. Click **No**.

Note: You will calculate statistics in "Step 4: Calculate Random Match Probabilities and Combined Probability of Inclusion/Exclusion."

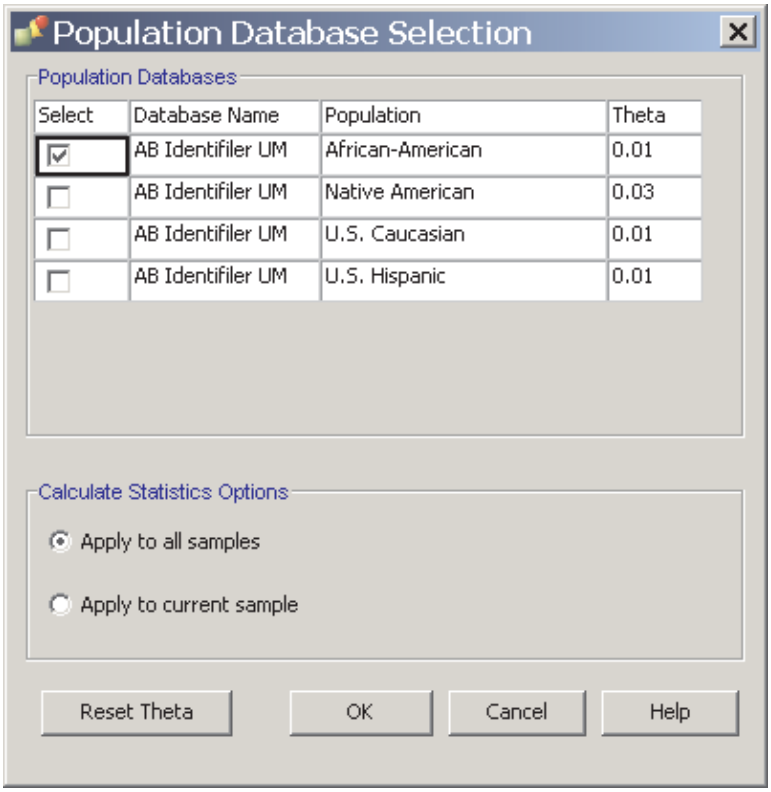
Review Remaining Samples

1. Click the **Next Sample>>** button to move to the next sample in the list. Review the data contained in both tables using the sample plot viewer.
2. Repeat for all remaining 2-contributor samples and remember to check **Sample Review Complete** following evaluation of each sample.

Note: You may observe during analysis of your own data that some samples do not produce any results following extraction. This is expected with samples containing a very low-level minor contribution where there are only 2-peak markers and no 3-peak or 4-peak markers.

Step 4: Calculate Random Match Probabilities and Combined Probability of Inclusion/Exclusion

1. In the Mixture Analysis Results Viewer, select **Sample Name: H4_H28_1_5** and click **Calculate Statistics** to display the Population Database Selection dialog box.



Note: For samples containing 2 contributors, the software performs the following statistical calculations: Random Match Probability (RMP) for each of the extracted contributor genotypes, Combined Probability of Inclusion/Exclusion (CPI/CPE), and Likelihood Ratio. Refer to Appendix A, "Statistical Analysis and Algorithms," and the *GeneMapper® ID-X Software Help* for additional information.

2. In the Population Database Selection dialog box, select the African-American population from the *Applied Biosystems Identifiler User Manual*, click the **Apply to all samples** radio buton, then click **OK** to view the results of the RMP and CPI/CPE statistical calculations for each sample.

   The RMP Statistics C1 (Major) tab opens by default. Data for the second contributor is found under the RMP Statistics C2 (Minor) tab.

   Continue to Step 5 to review the results.

Step 5: Review the Results of Random Match Probability and Combined Probability of Inclusion/Exclusion Calculations

1. Review the RMP data for the major contributor to sample H4_H28_1_5. Only one possible genotype per marker was selected for the major contributor.

2. Review the RMP data for the minor contributor for sample H4_H28_1_5.
   - For markers vWA, D2S1338, D18S51, D19S433 and TH01, marker dropout is represented by the F, F combination. For these markers, the software incorporates a value of 1.0 into the statistical calculation as shown in the example below.

RMP Statistics Details: Contributor 2(Minor)

	Database:Population	Marker	Alleles	Genotype Frequency	Combined Frequency
1	AB Identifiler UM:African-American	vWA	F,F	1.0000	1.0000
2	AB Identifiler UM:African-American	D16S539	9,9	0.0378	
3	AB Identifiler UM:African-American	D16S539	9,10	0.0416	
4	AB Identifiler UM:African-American	D16S539	9,12	0.0715	0.1510
5	AB Identifiler UM:African-American	D2S1338	F,F	1.0000	1.0000
6	AB Identifiler UM:African-American	D8S1179	10,13	0.0111	0.0111
7	AB Identifiler UM:African-American	D21S11	33.2,F	0.0756	0.0756

- For markers D3S1358, D21S11 and FGA, the software attributes a single allele to the minor contributor. For those markers, the software calculates a genotype frequency of 2p based on the detected allele frequency, and this is incorporated into the statistical calculation.

RMP Statistics Details: Contributor 2(Minor)

	Database:Population	Marker	Alleles	Genotype Frequency	Combined Frequency
5	AB Identifiler UM:African-American	D2S1338	F,F	1.0000	1.0000
6	AB Identifiler UM:African-American	D8S1179	10,13	0.0111	0.0111
7	AB Identifiler UM:African-American	D21S11	33.2,F	0.0756	0.0756
8	AB Identifiler UM:African-American	D18S51	F,F	1.0000	1.0000
9	AB Identifiler UM:African-American	D19S433	F,F	1.0000	1.0000
10	AB Identifiler UM:African-American	TH01	F,F	1.0000	1.0000
11	AB Identifiler UM:African-American	FGA	22,F	0.4314	0.4314

3. Click the **CPI/CPE Statistics** tab to review the results of the calculations.

4. Review the CPI/CPE results for sample H4_H28_1_5. In some instances, the software calculates that the possibility of allele dropout at a particular marker or markers is viable. For such markers, the software assigns the marker inclusion frequency a value of 1.0 based on the possibility that alleles may be present below the mixture interpretation threshold. Because the software includes all these potential alleles in the calculations, the marker is uninformative.

Step 6: Calculate and Review Likelihood Ratio Results

Note: If you are unfamiliar with Likelihood Ratio calculations, review Appendix A, "Statistical Analysis and Algorithms," before proceeding with this step.

To calculate the Likelihood Ratio, you must set up your alternate hypotheses by selecting the reference sample which, under the prosecution's hypothesis (H_p), cannot be excluded as the contributor of the evidence profile.

Click the **LR Statistics** tab. In this tab, you can:

- Define the hypotheses for calculating the Likelihood Ratio based on the extracted mixture results (selected genotype combinations) of both contributors to the mixture.
- Calculate a Likelihood Ratio based on a major contributor only. This approach can be used only when the minor contributor is uninterpretable and when the major component can be interpreted unambiguously.

Note: Refer to Appendix A, "Statistical Analysis and Algorithms," and the *GeneMapper® ID-X Software Help* for detailed statistics information.

Calculate the Likelihood Ratio Based on Contributors to the Mixture

The **Use (C1) Major Only** check box is deselected by default.

When this box is unselected, the software calculates Likelihood Ratios based on alternate hypotheses using both contributors to the mixture. The hypotheses available are based on whether one or two references are incorporated into the prosecution's hypothesis.

To calculate the Likelihood Ratio:

1. Select a single reference by choosing **Import...** from the Reference 1 drop-down menu and navigating to the Known_H28.txt file you saved earlier in Chapter 3, "Step 5: Export and View a Known Genotype Profile" on page 35.

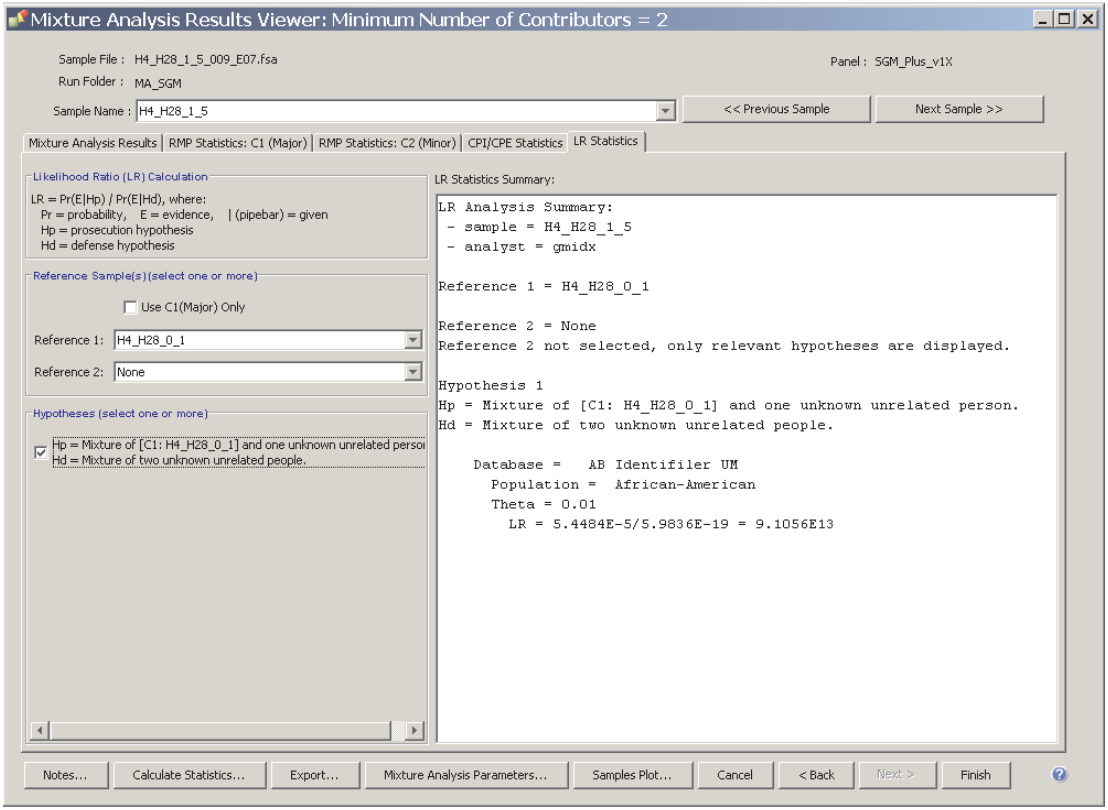
   Note: When you select the reference, it does not display the exported file name. Instead, it displays the original sample name taken from the sample file.

   IMPORTANT! If you import a new known genotype containing the same name as one that currently exists in the Mixture Analysis tool and there are marker conflicts (same name, different alleles), no markers from the new known genotype profile are imported (the existing known genotype profile remains unchanged). If there are no marker conflicts (same name, same alleles), then all markers from the new known genotype profile that do not occur in the existing known genotype profile are added to that profile.

2. In the Known Genotypes dialog box, click **OK**.

   When you select a single reference, one hypothesis is displayed.

3. Check the box to the left of the hypothesis to display the Likelihood Ratio Statistics Summary, as shown next.


Mixture Analysis Results Viewer: Minimum Number of Contributors = 2
Sample File : H4_H28_1_5_009_E07.fsa
Panel : SGM_Plus_v1X
Run Folder : MA_SGM
Sample Name : H4_H28_1_5
<< Previous Sample
Next Sample >>
Mixture Analysis Results
RMP Statistics: C1 (Major)
RMP Statistics: C2 (Minor)
CPI/CPE Statistics
LR Statistics
Likelihood Ratio (LR) Calculation
LR = Pr(E|Hp) / Pr(E|Hd), where:
Pr = probability, E = evidence, | (pipebar) = given
Hp = prosecution hypothesis
Hd = defense hypothesis
Reference Sample(s) (select one or more)
Use C1(Major) Only
Reference 1: H4_H28_0_1
Reference 2: None
Hypotheses (select one or more)
Hp = Mixture of [C1: H4_H28_0_1] and one unknown unrelated perso
Hd = Mixture of two unknown unrelated people.
LR Statistics Summary:
LR Analysis Summary:
- sample = H4_H28_1_5
- analyst = gmidx
Reference 1 = H4_H28_0_1
Reference 2 = None
Reference 2 not selected, only relevant hypotheses are displayed.
Hypothesis 1
Hp = Mixture of [C1: H4_H28_0_1] and one unknown unrelated person.
Hd = Mixture of two unknown unrelated people.
Database = AB Identifiler UM
Population = African-American
Theta = 0.01
LR = 5.4484E-5/5.9836E-19 = 9.1056E13
Notes...
Calculate Statistics...
Export...
Mixture Analysis Parameters...
Samples Plot...
Cancel
< Back
Next >
Finish

The software calculates the Likelihood Ratio based on the following alternate hypotheses:

- H_p = H4_H28_1_5 originates from a mixture of H28 and one unknown, unrelated person
- H_d = H4_H28_1_5 originates from a mixture of two unknown, unrelated people

The observed profile is 9.1056E13 or 91 trillion times more likely to occur if it originated from reference H28 and one unknown unrelated person than from a mixture of two unknown unrelated people.

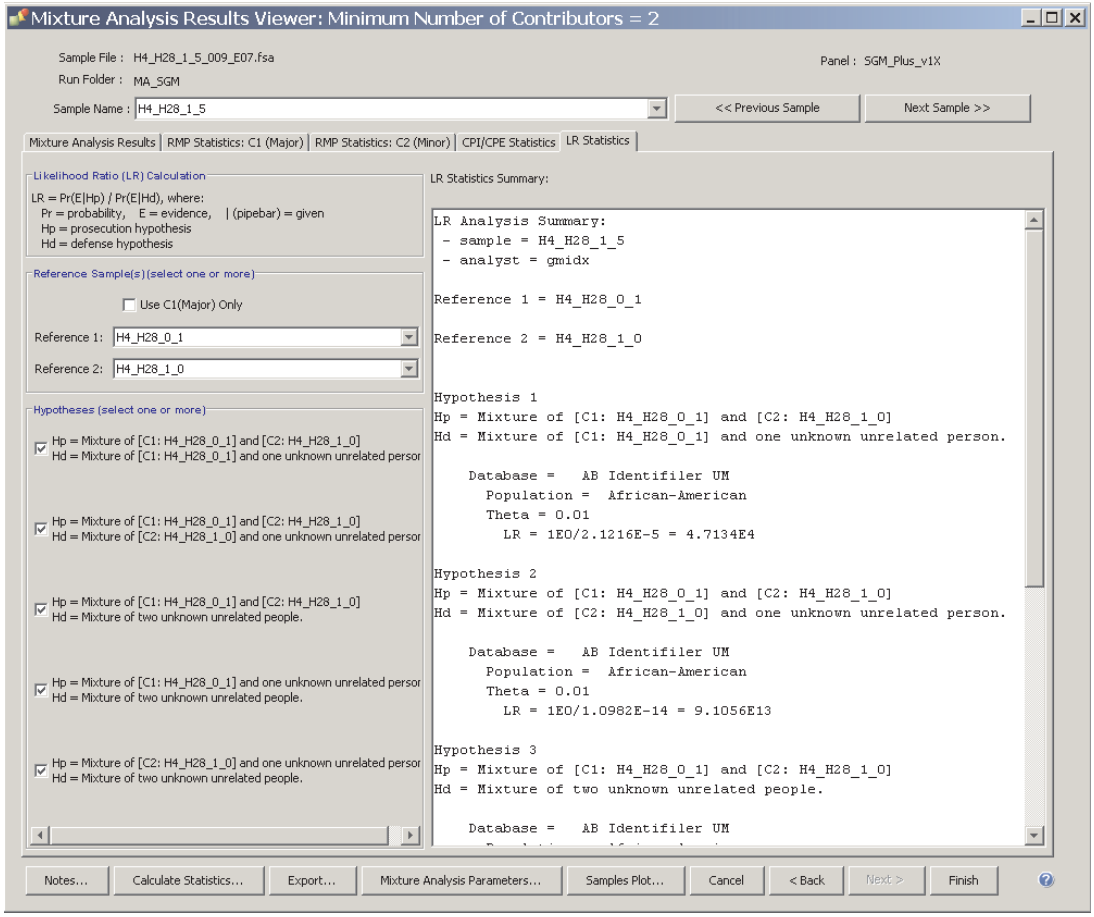
You can also calculate a Likelihood Ratio based on the hypothesis that both contributors to the mixture are known.

4. Select a second reference: In the Reference 2: drop-down field click **Import…**, navigate to the location where you saved the *Known_H4 .txt* file, as shown in Chapter 3 "Step 5: Export and View a Known Genotype Profile" on page 35, then select **Import**.

5. In the Known Genotypes dialog box, click **OK**.

   When you select two references, multiple hypotheses are displayed.

6. Check the boxes to the left of the hypotheses to display the Likelihood Ratio Statistics Summary, as shown next.


Mixture Analysis Results Viewer: Minimum Number of Contributors = 2
Sample File : H4_H28_1_5_009_E07.fsa
Panel : SGM_Plus_v1X
Run Folder : MA_SGM
Sample Name : H4_H28_1_5
<< Previous Sample
Next Sample >>
Mixture Analysis Results
RMP Statistics: C1 (Major)
RMP Statistics: C2 (Minor)
CPI/CPE Statistics
LR Statistics
Likelihood Ratio (LR) Calculation
LR = Pr(E|Hp) / Pr(E|Hd), where:
Pr = probability, E = evidence, | (pipebar) = given
Hp = prosecution hypothesis
Hd = defense hypothesis
Reference Sample(s) (select one or more)
Use C1(Major) Only
Reference 1: H4_H28_0_1
Reference 2: H4_H28_1_0
Hypotheses (select one or more)
Hp = Mixture of [C1: H4_H28_0_1] and [C2: H4_H28_1_0]
Hd = Mixture of [C1: H4_H28_0_1] and one unknown unrelated person
Hp = Mixture of [C1: H4_H28_0_1] and [C2: H4_H28_1_0]
Hd = Mixture of [C2: H4_H28_1_0] and one unknown unrelated person
Hp = Mixture of [C1: H4_H28_0_1] and [C2: H4_H28_1_0]
Hd = Mixture of two unknown unrelated people.
Hp = Mixture of [C1: H4_H28_0_1] and one unknown unrelated person
Hd = Mixture of two unknown unrelated people.
Hp = Mixture of [C2: H4_H28_1_0] and one unknown unrelated person
Hd = Mixture of two unknown unrelated people.
LR Statistics Summary:
LR Analysis Summary:
- sample = H4_H28_1_5
- analyst = gmidx
Reference 1 = H4_H28_0_1
Reference 2 = H4_H28_1_0
Hypothesis 1
Hp = Mixture of [C1: H4_H28_0_1] and [C2: H4_H28_1_0]
Hd = Mixture of [C1: H4_H28_0_1] and one unknown unrelated person.
Database = AB Identifiler UM
Population = African-American
Theta = 0.01
LR = 1EO/2.1216E-5 = 4.7134E4
Hypothesis 2
Hp = Mixture of [C1: H4_H28_0_1] and [C2: H4_H28_1_0]
Hd = Mixture of [C2: H4_H28_1_0] and one unknown unrelated person.
Database = AB Identifiler UM
Population = African-American
Theta = 0.01
LR = 1EO/1.0982E-14 = 9.1056E13
Hypothesis 3
Hp = Mixture of [C1: H4_H28_0_1] and [C2: H4_H28_1_0]
Hd = Mixture of two unknown unrelated people.
Database = AB Identifiler UM
Notes...
Calculate Statistics...
Export...
Mixture Analysis Parameters...
Samples Plot...
Cancel
< Back
Next >
Finish

The software calculates the Likelihood Ratio based on the following alternate hypotheses.

Hypothesis 1

- H_p = H4_H28_1_5 originates from a mixture of H28 and H4
- H_d = H4_H28_1_5 originates from a mixture of H28 and one unknown unrelated person.

  The observed profile is 4.7134E4 or 47000 times more likely to occur if it originated from a mixture of H28 and H24 than from a mixture of H28 and one unknown unrelated person.

Hypothesis 2

- H_p = H4_H28_1_5 originates from a mixture of H28 and H24
- H_d = H4_H28_1_5 originates from a mixture of H4 and one unknown unrelated person

  The observed profile is 9.1056E13 or
  91 trillion times more likely to occur if it originated from a mixture of H28 and H24 than from a mixture of H4 and one unknown unrelated person.

Hypothesis 3

- H_p = H4_H28_1_5 originates from a mixture of H28 and H24
- H_d = H4_H28_1_5 originates from a mixture of two unknown unrelated people

  The observed profile is 4.2918E18 or 4.3 quintillion times more likely to occur if it originated from mixture of H28 and H24 than from a mixture of two unknown unrelated people.

Hypothesis 4

- H_p = H4_H28_1_5 originates from a mixture of H4 and one unknown unrelated person
- H_d = H4_H28_1_5 originates from a mixture of two unknown unrelated people

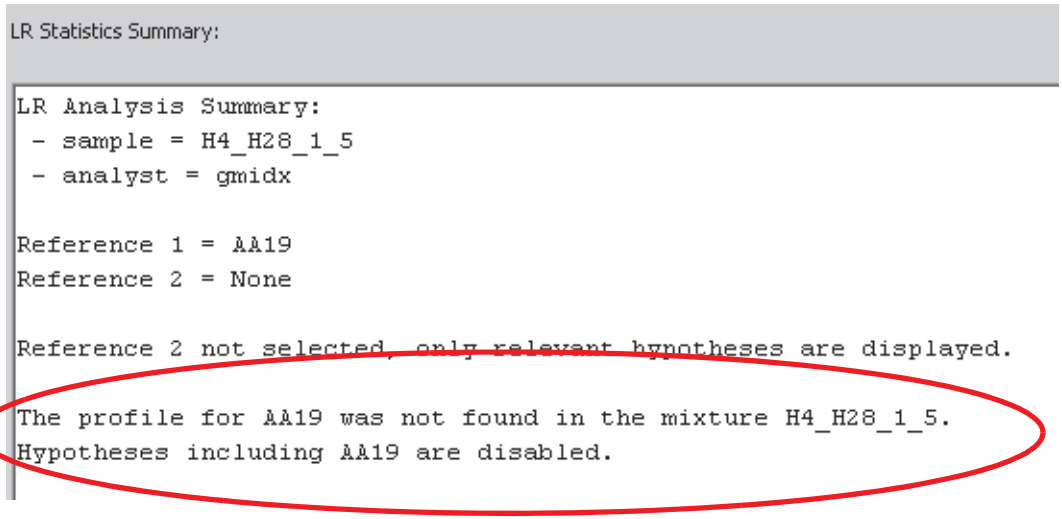
  The observed profile is 4.7134E4 or 47000 times more likely to occur if it originated from reference H4 and one unknown unrelated person than from a mixture of two unknown unrelated people.

Hypothesis 5

- H_p = H4_H28_1_5 originates from a mixture of H28 and one unknown unrelated person
- H_d = H4_H28_1_5 originates from a mixture of two unknown unrelated people

  The observed profile is 9.1056E13 or 91 trillion times times more likely to occur if it originated from reference H28 and one unknown unrelated person than from a mixture of two unknown unrelated people.

For all hypotheses, the software validates the inclusion of the reference profile in the mixture profile. If you select a reference that cannot be included in the mixture based on comparison of the contributor genotypes, the software displays a message that the validation of inclusion of the reference profile in the mixture failed, as shown next.

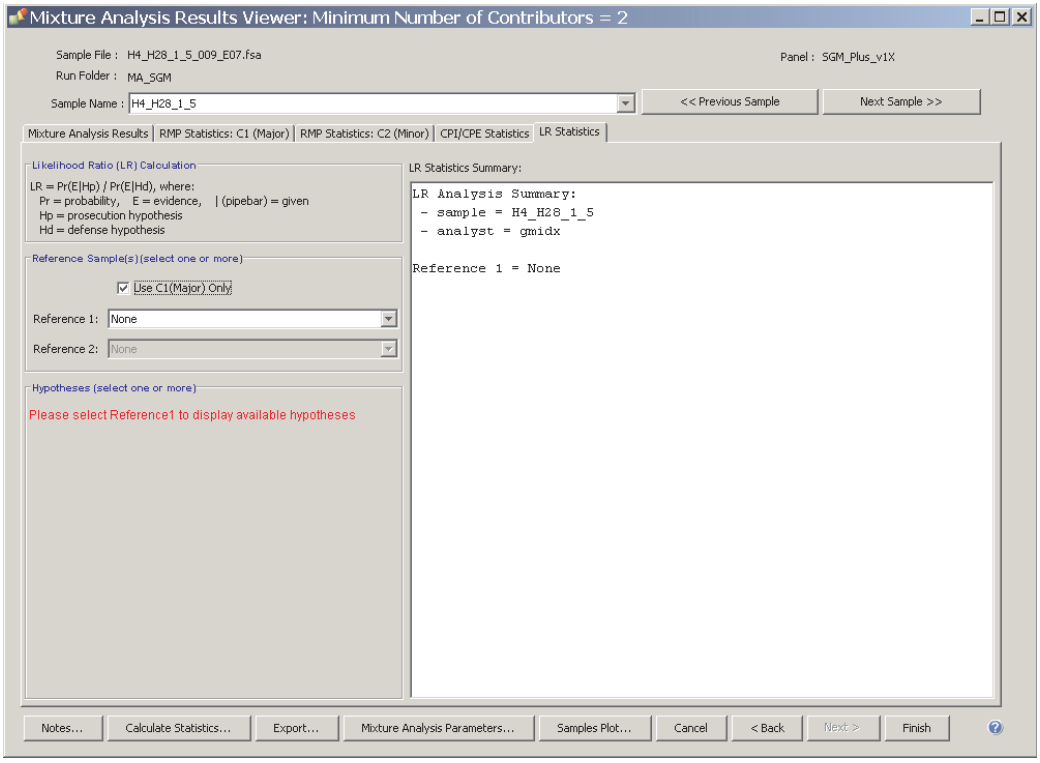

LR Statistics Summary:
LR Analysis Summary:
- sample = H4_H28_1_5
- analyst = gmidx
Reference 1 = AA19
Reference 2 = None
Reference 2 not selected, only relevant hypotheses are displayed.
The profile for AA19 was not found in the mixture H4_H28_1_5.
Hypotheses including AA19 are disabled.

1. Click **<Back** to return to the Mixture Analysis Sample Segregation dialog box.
2. If desired, click **Yes** to save the changes to the current sample.

IMPORTANT! Saved extraction results are overwritten when you reanalyze a sample.

Calculate the Likelihood Ratio Based on the Major Contributor Only

1. To calculate the Likelihood Ratio for a major contributor only, check the **Use (C1) Major Only** box as shown next.

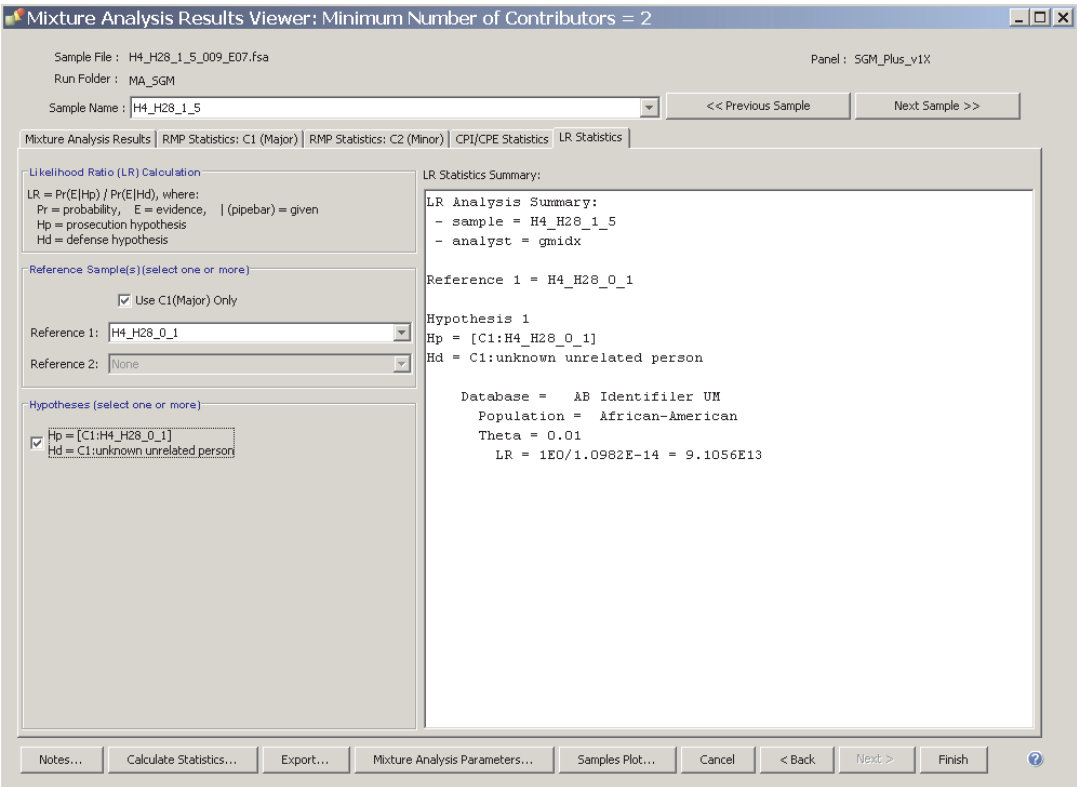


2. In the Reference 1: drop-down field click **Import…**, navigate to the location where you saved the *Known_H28.txt* file shown in Chapter 3 “Step 5: Export and View a Known Genotype Profile” on page 35, then select **Import**.

3. In the Known Genotypes dialog box, click **OK**.

   One hypothesis is displayed.

4. Check the box to the left of the hypothesis to display the Likelihood Ratio Statistics Summary, as shown next.



The software calculates the Likelihood Ratio based on the following alternate hypotheses:

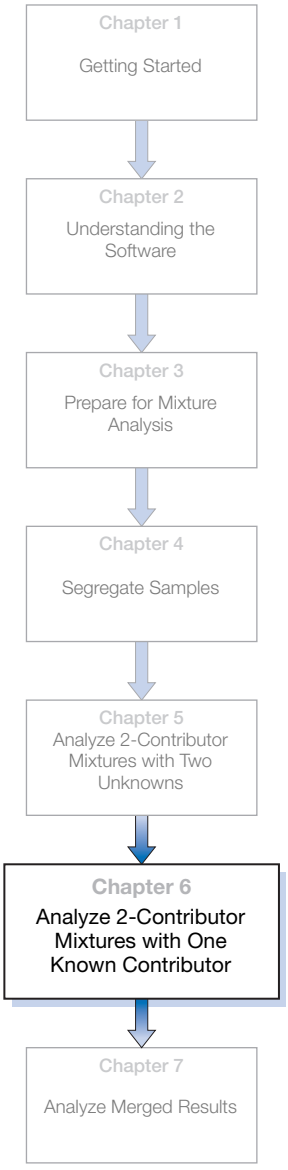
- H_p = Reference H28 is the source of the major contributor DNA
- H_d = An unknown contributor is the source of the major contributor DNA

In this example the observed profile is 9.1056E13 or 91 trillion times more likely to occur if it originated from reference H28 than from an unknown unrelated African-American individual.

Note: The Likelihood Ratio Summary displays only a brief description of the LR calculations. To view detailed LR calculations, select **Export...**, then **LR Statistics**. Save the exported report as a comma-separated values file (*.csv). The exported report contains the details for each population calculated using the likelihood ratio.

Refer to the *GeneMapper® ID-X Software Help* for additional information on segregating samples and extracting individuals from 2-contributor mixtures.

6 Analyze 2-Contributor Mixtures with One Known Contributor



This chapter covers:

- Overview. 86
- Step 1: Assign Known Genotypes 86
- Step 2: Calculate the Random Match Probability for 2-Contributor Mixtures Extracted with a Known Contributor. . 92
- Step 3: Calculate the Likelihood Ratio for 2-Contributor Mixtures Extracted with a Known Contributor 94

Overview

About Analysis of 2-Contributor Samples Where 1 contributor is Known

In this chapter, you will repeat the workflow described in Chapter 5, "Analyze 2-Contributor Mixtures with Two Unknowns," but this time a known reference will be used to further refine the selected genotype combinations.

You can use this method when one known contributor to an evidence sample can be reasonably assumed to be present. For example, from an intimate vaginal sample, it is reasonable to assume that the DNA taken from the female is present on the swab. In such cases, the known contributor is "subtracted" from the selected genotype combinations following extraction, leaving the possible profiles for the unknown contributor.

In This Chapter

In this chapter, you will learn to:

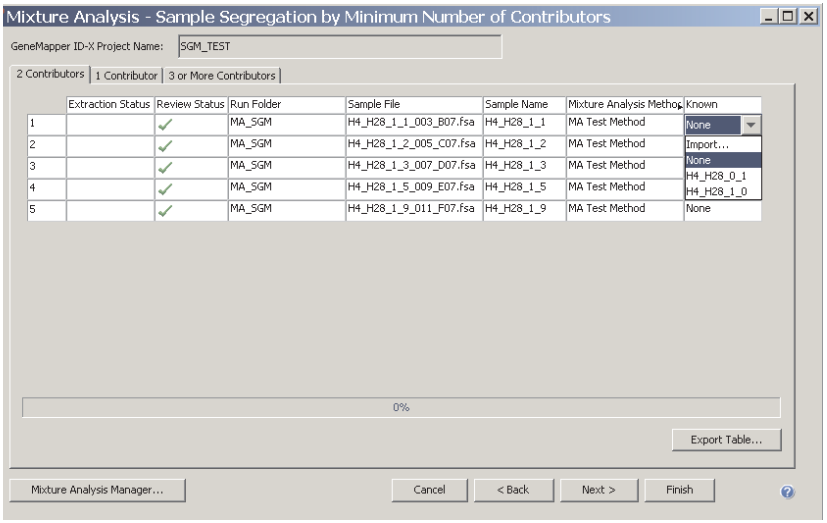
- Assign known genotypes.
- Extract individual genotypes of two possible contributors of a two-person mixture where 1 contributor is known.
- Determine which genotype combinations to include in the statistical calculations.
- Calculate Random Match Probability (RMP) for the unknown contributor.
- Calculate and review Likelihood Ratio results.

Step 1: Assign Known Genotypes

Select Known Contributors

1. Select the **2 Contributors** tab in the Mixture Analysis - Sample Segregation dialog box. After conducting the review processes in Chapter 5 "Step 2: Review the Results of the Extraction" on page 58, the Review Status column should indicate that all samples were reviewed.

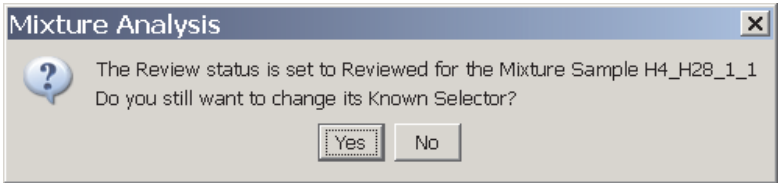
2. Click in the Known column adjacent to sample H4_H28_1_1 and select H4_H28_1_0 from the drop-down list. This sample was previously imported in Chapter 5 "Step 6: Calculate and Review Likelihood Ratio Results" on page 74.



or

To import another known sample click **Import…**, then from the drop-down list navigate to the location of a different known genotype profile and click **Import**.

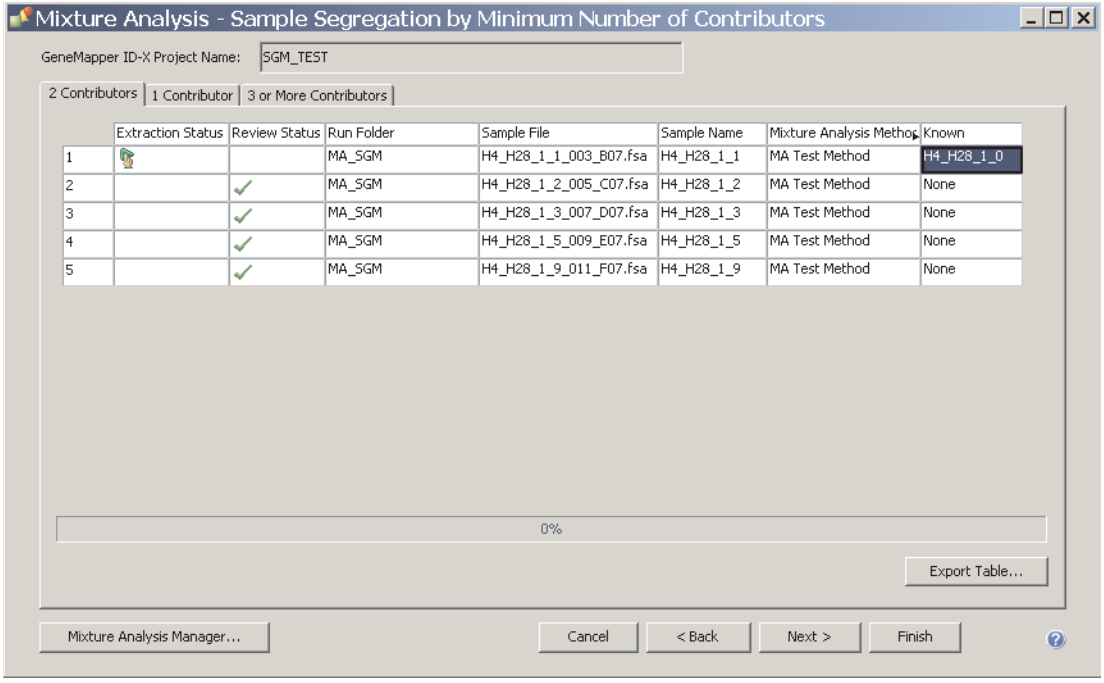
The following message is displayed indicating that the review status will change if you select a known contributor.


Mixture Analysis
The Review status is set to Reviewed for the Mixture Sample H4_H28_1_1
Do you still want to change its Known Selector?
Yes
No

Note: This message is displayed only if you previously reviewed a sample and checked **Sample Review Complete** where no known contributor was used, but then went back and selected a known contributor to include during extraction.

3. Click **Yes**.

4. The sample is displayed in the Known column of the Mixture Analysis - Sample Segregation by Minimum Number of Contributors table as shown.



Note: A green ( ) arrow appears in the Extraction Status column for sample H4_H28_1_1 indicating a change to the mixture analysis criteria. Previous results will be lost unless you click **No** before proceeding with known genotype selection.

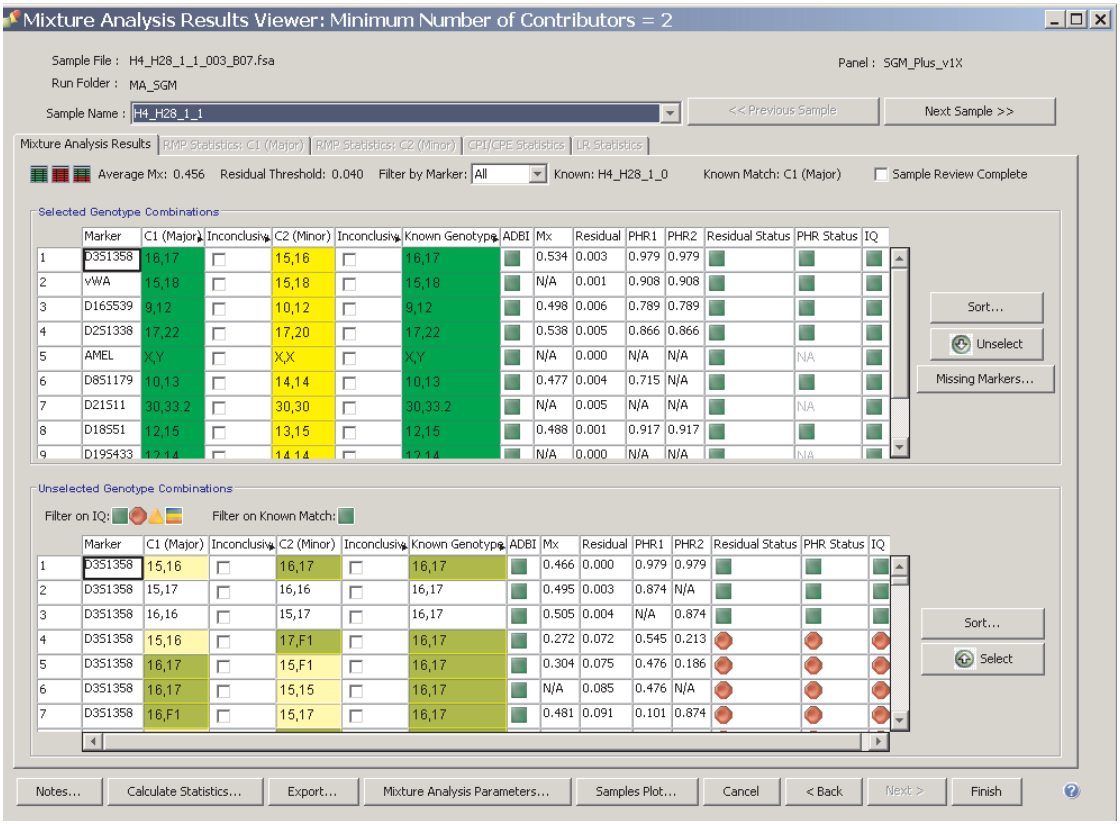
5. Select the **Known** column header to highlight the column, press **Ctrl-D** to apply (fill-down) the known to all samples, then click **Yes** when the Review status message is displayed for each sample.

6. Click **Next >** to proceed with extraction of the mixtures with consideration of the selected known contributor.

   The Mixture Analysis Results Viewer displays sample H4_H28_1_1.

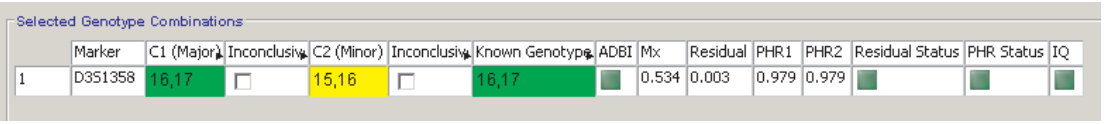
Review Extraction Results

1. Review the Selected Genotype Combinations table. The bright green highlighting indicates that the known genotype profile exactly matches (across all markers) the extracted genotype combinations of 1 contributor to the mixture. The bright yellow highlighting indicates the extracted genotype combinations of the unknown contributor to the mixture. The software uses the known genotypes to further filter the table, and only displays combinations that have passing IQ status and contain the known genotype.


Mixture Analysis Results Viewer: Minimum Number of Contributors = 2
Sample File : H4_H28_1_1_003_B07.fsa
Panel : SGM_Plus_v1X
Run Folder : MA_SGM
Sample Name : H4_H28_1_1
<< Previous Sample
Next Sample >>
Mixture Analysis Results
RMP Statistics: C1 (Major)
RMP Statistics: C2 (Minor)
CPI/CPE Statistics
LR Statistics
Average Mx: 0.456
Residual Threshold: 0.040
Filter by Marker: All
Known: H4_H28_1_0
Known Match: C1 (Major)
Sample Review Complete
Selected Genotype Combinations
Marker | C1 (Major) | Inconclusive | C2 (Minor) | Inconclusive | Known Genotype | ADBI | Mx | Residual | PHR1 | PHR2 | Residual Status | PHR Status | IQ
1 D3S1358 16,17 15,16 16,17 0.534 0.003 0.979 0.979
2 vWA 15,18 15,18 15,18 N/A 0.001 0.908 0.908
3 D16S539 9,12 10,12 9,12 0.498 0.006 0.789 0.789
4 D2S1338 17,22 17,20 17,22 0.538 0.005 0.866 0.866
5 AMEL X,Y X,X X,Y N/A 0.000 N/A N/A NA
6 D8S1179 10,13 14,14 10,13 0.477 0.004 0.715 N/A
7 D21S11 30,33.2 30,30 30,33.2 N/A 0.005 N/A N/A NA
8 D18S51 12,15 13,15 12,15 0.488 0.001 0.917 0.917
9 D19S433 12,14 14,14 12,14 N/A 0.000 N/A N/A NA
Sort...
Unselect
Missing Markers...
Unselected Genotype Combinations
Filter on IQ:
Filter on Known Match:
Marker | C1 (Major) | Inconclusive | C2 (Minor) | Inconclusive | Known Genotype | ADBI | Mx | Residual | PHR1 | PHR2 | Residual Status | PHR Status | IQ
1 D3S1358 15,16 16,17 16,17 0.466 0.000 0.979 0.979
2 D3S1358 15,17 16,16 16,17 0.495 0.003 0.874 N/A
3 D3S1358 16,16 15,17 16,17 0.505 0.004 N/A 0.874
4 D3S1358 15,16 17,F1 16,17 0.272 0.072 0.545 0.213
5 D3S1358 16,17 15,F1 16,17 0.304 0.075 0.476 0.186
6 D3S1358 16,17 15,15 16,17 N/A 0.085 0.476 N/A
7 D3S1358 16,F1 15,17 16,17 0.481 0.091 0.101 0.874
Sort...
Select
Notes...
Calculate Statistics...
Export...
Mixture Analysis Parameters...
Samples Plot...
Cancel
< Back
Next >
Finish

2. In the previous example, review sample H4_H28_1_1 and select **Filter by Marker: D3S1358**.

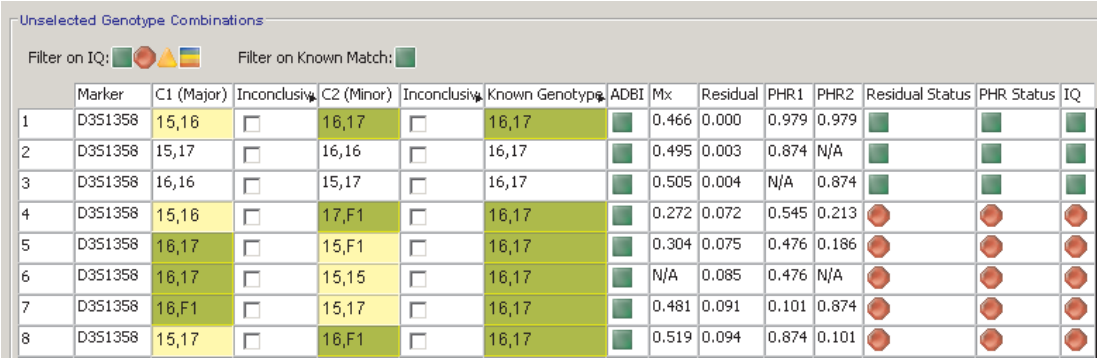
The C1 (Major) contributor in the Selected Genotype Combinations table is a 16, 17 (as noted below) and matches the known whereas the C2 (Minor) contributor is a 15, 16.



Selected Genotype Combinations

	Marker	C1 (Major)	Inconclusiv	C2 (Minor)	Inconclusiv	Known Genotype	ADBI	Mx	Residual	PHR1	PHR2	Residual Status	PHR Status	IQ
1	D3S1358	16,17	☐	15,16	☐	16,17	■	0.534	0.003	0.979	0.979	■	■	■

Note: In the Unselected Genotype Combinations table there are three genotype pairs that pass all PQV metrics (Residual Status, PHR Status and IQ) and yet are not included in the Selected Genotype Combinations table (shown below). Patterns 2 and 3 do not contain a genotype that matches the known contributor and, therefore, do not meet the criteria for known filtering. Pattern 1 is the inverse (Major 15, 16 and Minor 16, 17) of the selected pattern (Major 16, 17 and Minor 15, 16). In approximate 1:1 mixtures where both patterns have high Inclusion Quality, the known selection always displays the match to the C1 (Major) contributor.



Unselected Genotype Combinations

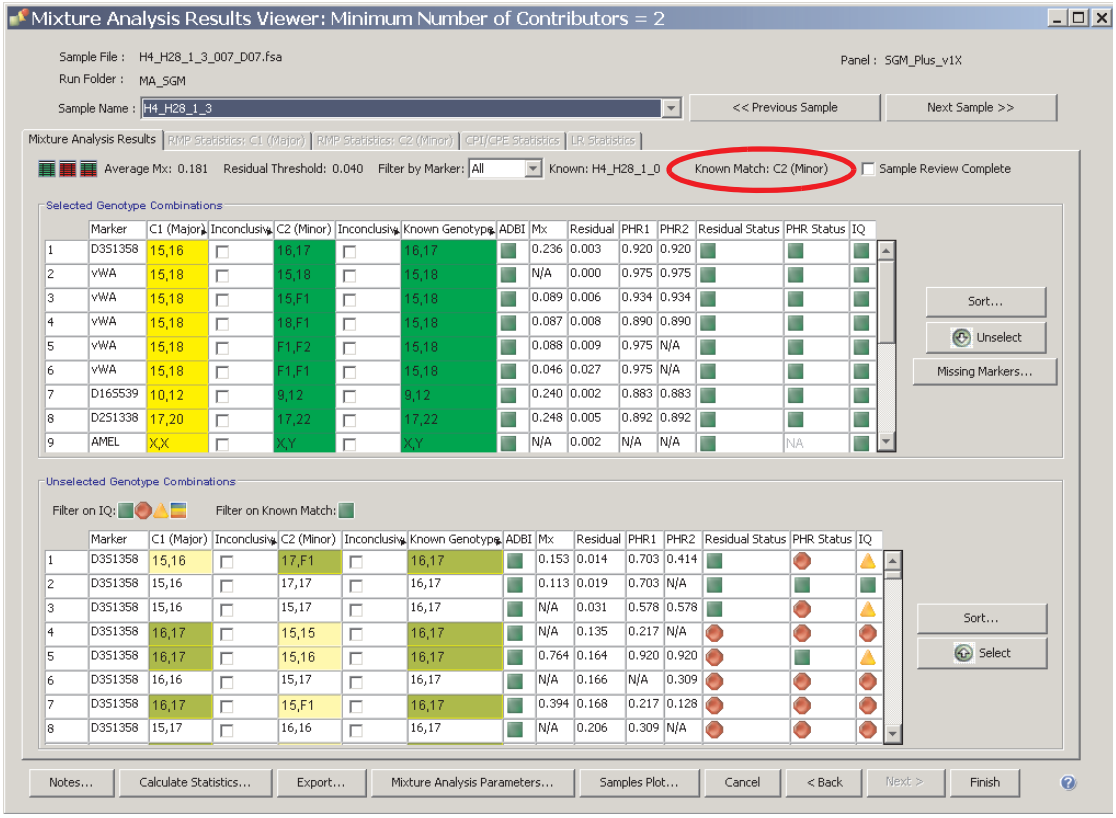
Filter on IQ: ■ ● ▲ ■ Filter on Known Match: ■

	Marker	C1 (Major)	Inconclusiv	C2 (Minor)	Inconclusiv	Known Genotype	ADBI	Mx	Residual	PHR1	PHR2	Residual Status	PHR Status	IQ
1	D3S1358	15,16	☐	16,17	☐	16,17	■	0.466	0.000	0.979	0.979	■	■	■
2	D3S1358	15,17	☐	16,16	☐	16,17	■	0.495	0.003	0.874	N/A	■	■	■
3	D3S1358	16,16	☐	15,17	☐	16,17	■	0.505	0.004	N/A	0.874	■	■	■
4	D3S1358	15,16	☐	17,F1	☐	16,17	■	0.272	0.072	0.545	0.213	●	●	●
5	D3S1358	16,17	☐	15,F1	☐	16,17	■	0.304	0.075	0.476	0.186	●	●	●
6	D3S1358	16,17	☐	15,15	☐	16,17	■	N/A	0.085	0.476	N/A	●	●	●
7	D3S1358	16,F1	☐	15,17	☐	16,17	■	0.481	0.091	0.101	0.874	●	●	●
8	D3S1358	15,17	☐	16,F1	☐	16,17	■	0.519	0.094	0.874	0.101	●	●	●

Note: In instances where markers are missing from the Selected Genotype Combinations table and the entire genotype cannot be attributed solely to 1 contributor, the highlighting is light yellow and light green. In such cases, click the **Missing Markers…** button, then review the Unselected Genotype Combinations table to display the missing markers.

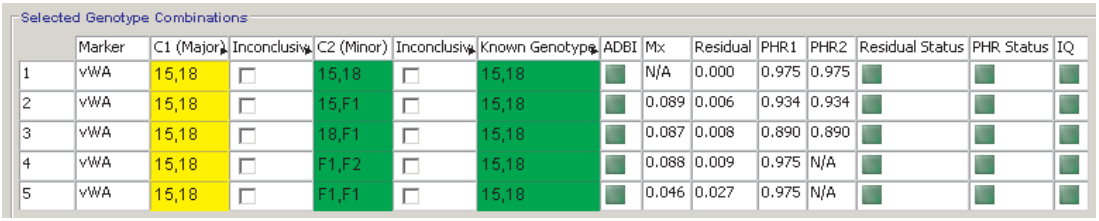
3. In this example, review sample H4_H28_1_3.

   The known contributor matches the C2 (Minor) contributor completely.


Mixture Analysis Results Viewer: Minimum Number of Contributors = 2
Sample File : H4_H28_1_3_007_D07.fsa
Panel : SGM_Plus_v1X
Run Folder : MA_SGM
Sample Name : H4_H28_1_3
<< Previous Sample
Next Sample >>
Mixture Analysis Results
RMP Statistics: C1 (Major)
RMP Statistics: C2 (Minor)
GPI/CPE Statistics
LR Statistics
Average Mx: 0.181
Residual Threshold: 0.040
Filter by Marker: All
Known: H4_H28_1_0
Known Match: C2 (Minor)
Sample Review Complete
Selected Genotype Combinations
Marker | C1 (Major) | Inconclusive | C2 (Minor) | Inconclusive | Known Genotype | ADBI | Mx | Residual | PHR1 | PHR2 | Residual Status | PHR Status | IQ
1 D3S1358 15,16 16,17 16,17 0.236 0.003 0.920 0.920
2 vWA 15,18 15,18 15,18 N/A 0.000 0.975 0.975
3 vWA 15,18 15,F1 15,18 0.089 0.006 0.934 0.934
4 vWA 15,18 18,F1 15,18 0.087 0.008 0.890 0.890
5 vWA 15,18 F1,F2 15,18 0.088 0.009 0.975 N/A
6 vWA 15,18 F1,F1 15,18 0.046 0.027 0.975 N/A
7 D16S539 10,12 9,12 9,12 0.240 0.002 0.883 0.883
8 D2S1338 17,20 17,22 17,22 0.248 0.005 0.892 0.892
9 AMEL X,X X,Y X,Y N/A 0.002 N/A N/A NA
Sort...
Unselect
Missing Markers...
Unselected Genotype Combinations
Filter on IQ:
Filter on Known Match:
Marker | C1 (Major) | Inconclusive | C2 (Minor) | Inconclusive | Known Genotype | ADBI | Mx | Residual | PHR1 | PHR2 | Residual Status | PHR Status | IQ
1 D3S1358 15,16 17,F1 16,17 0.153 0.014 0.703 0.414
2 D3S1358 15,16 17,17 16,17 0.113 0.019 0.703 N/A
3 D3S1358 15,16 15,17 16,17 N/A 0.031 0.578 0.578
4 D3S1358 16,17 15,15 16,17 N/A 0.135 0.217 N/A
5 D3S1358 16,17 15,16 16,17 0.764 0.164 0.920 0.920
6 D3S1358 16,16 15,17 16,17 N/A 0.166 N/A 0.309
7 D3S1358 16,17 15,F1 16,17 0.394 0.168 0.217 0.128
8 D3S1358 15,17 16,16 16,17 N/A 0.206 0.309 N/A
Sort...
Select
Notes...
Calculate Statistics...
Export...
Mixture Analysis Parameters...
Samples Plot...
Cancel
< Back
Next >
Finish

4. Select **Filter by Marker: vWA**.

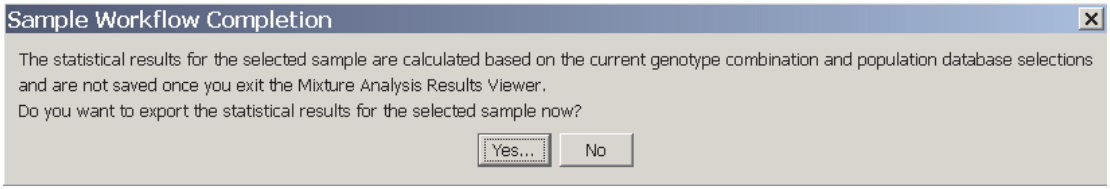
   The five genotype combinations are listed in the Selected Genotype Combinations table. In all cases the C1(Major) contributor is a type 15, 18 whereas four different possible genotypes are listed for the C2 (Minor) contributor: 15, 18; 15, F1; 18, F1; F1, F1; and F1, F2. The known 15, 18 contributor is not excluded from any of these possibilities.



Selected Genotype Combinations

	Marker	C1 (Major)	Inconclusive	C2 (Minor)	Inconclusive	Known Genotype	ADBI	Mx	Residual	PHR1	PHR2	Residual Status	PHR Status	IQ
1	vWA	15,18	☐	15,18	☐	15,18		N/A	0.000	0.975	0.975			
2	vWA	15,18	☐	15,F1	☐	15,18		0.089	0.006	0.934	0.934			
3	vWA	15,18	☐	18,F1	☐	15,18		0.087	0.008	0.890	0.890			
4	vWA	15,18	☐	F1,F2	☐	15,18		0.088	0.009	0.975	N/A			
5	vWA	15,18	☐	F1,F1	☐	15,18		0.046	0.027	0.975	N/A			

5. Check the **Sample Review Complete** box to indicate that this sample is ready for statistical analysis. The Sample Workflow Completion dialog box is displayed.


Sample Workflow Completion
The statistical results for the selected sample are calculated based on the current genotype combination and population database selections and are not saved once you exit the Mixture Analysis Results Viewer.
Do you want to export the statistical results for the selected sample now?
Yes...
No

6. Click **No.**

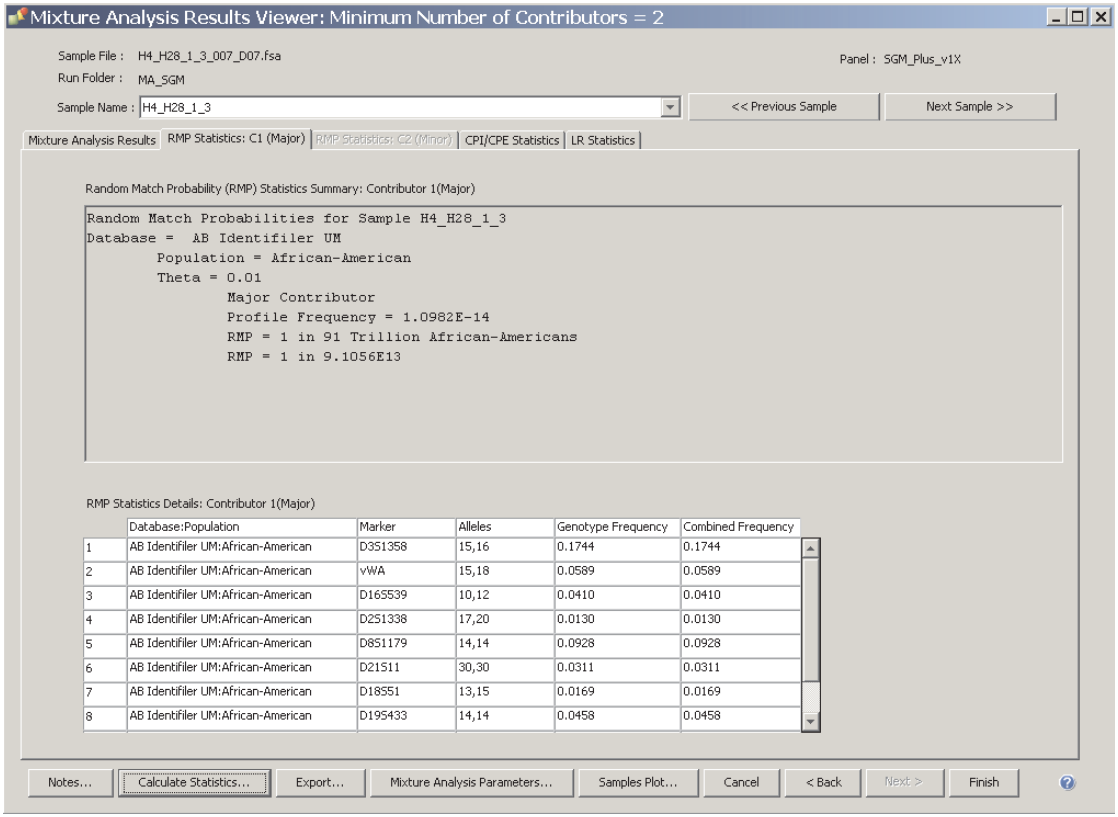
Note: You will calculate statistics in "Step 2: Calculate the Random Match Probability for 2-Contributor Mixtures Extracted with a Known Contributor."

7. Select **Next Sample >>** to review the next sample extracted with the known.

Step 2: Calculate the Random Match Probability for 2-Contributor Mixtures Extracted with a Known Contributor

1. Click **Calculate Statistics**.
2. In the Population Database dialog box, select the African-American population for the *Applied Biosystems Identifiler User Manual* database, apply it to the current sample, then click **OK**.

3. Verify and note that the RMP statistic is only calculated for the unknown C1 (Major) contributor.

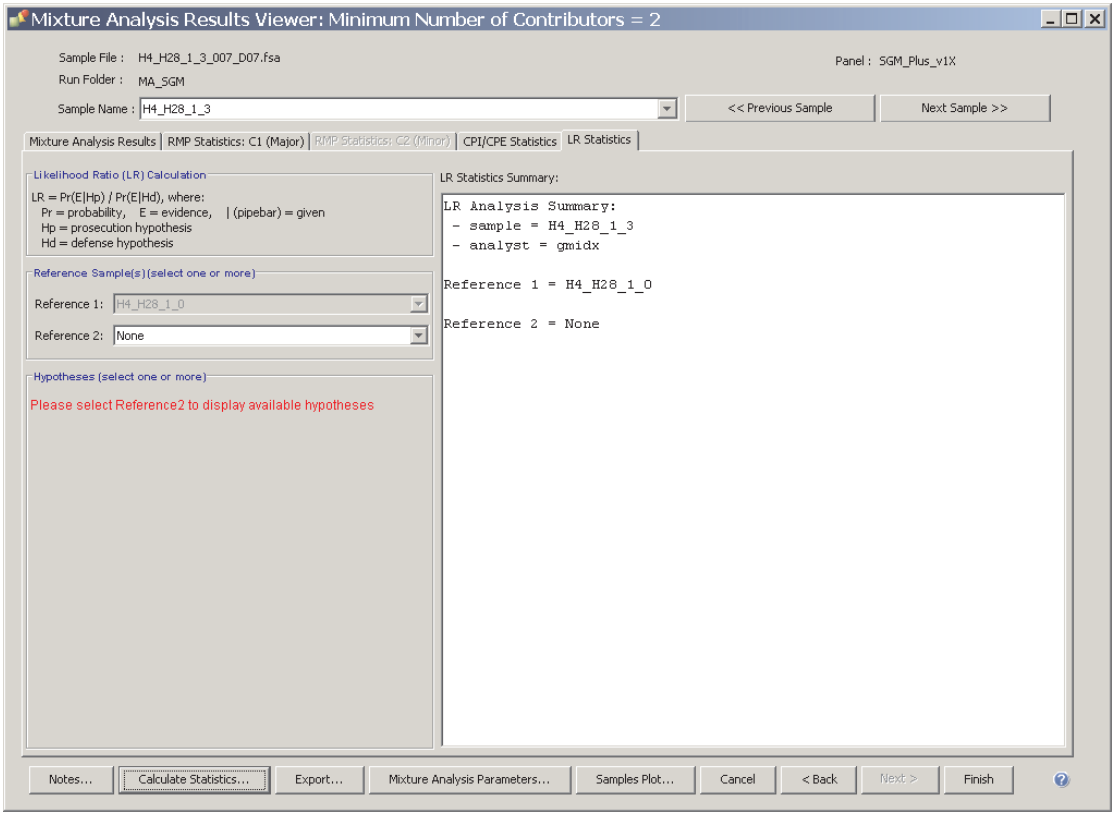

Mixture Analysis Results Viewer: Minimum Number of Contributors = 2
Sample File : H4_H28_1_3_007_D07.fsa
Run Folder : MA_SGM
Panel : SGM_Plus_v1X
Sample Name : H4_H28_1_3
<< Previous Sample
Next Sample >>
Mixture Analysis Results
RMP Statistics: C1 (Major)
RMP Statistics: C2 (Minor)
CPI/CPE Statistics
LR Statistics
Random Match Probability (RMP) Statistics Summary: Contributor 1(Major)
Random Match Probabilities for Sample H4_H28_1_3
Database = AB Identifiler UM
Population = African-American
Theta = 0.01
Major Contributor
Profile Frequency = 1.0982E-14
RMP = 1 in 91 Trillion African-Americans
RMP = 1 in 9.1056E13
RMP Statistics Details: Contributor 1(Major)
Database:Population
Marker
Alleles
Genotype Frequency
Combined Frequency
1 AB Identifiler UM:African-American D3S1358 15,16 0.1744 0.1744
2 AB Identifiler UM:African-American vWA 15,18 0.0589 0.0589
3 AB Identifiler UM:African-American D16S539 10,12 0.0410 0.0410
4 AB Identifiler UM:African-American D2S1338 17,20 0.0130 0.0130
5 AB Identifiler UM:African-American D8S1179 14,14 0.0928 0.0928
6 AB Identifiler UM:African-American D21S11 30,30 0.0311 0.0311
7 AB Identifiler UM:African-American D18S51 13,15 0.0169 0.0169
8 AB Identifiler UM:African-American D19S433 14,14 0.0458 0.0458
Notes...
Calculate Statistics...
Export...
Mixture Analysis Parameters...
Samples Plot...
Cancel
< Back
Next >
Finish

4. Select the **CPI/CPE Statistics** tab to display the Combined Probability of Inclusion/Exclusion. This statistic incorporates the entire complement of alleles present and does not change when you use a known contributor in the extraction process.

Step 3: Calculate the Likelihood Ratio for 2-Contributor Mixtures Extracted with a Known Contributor

1. Select the **LR Statistics** tab.

   Note that the known reference that you used in the extraction is already selected as Reference 1.

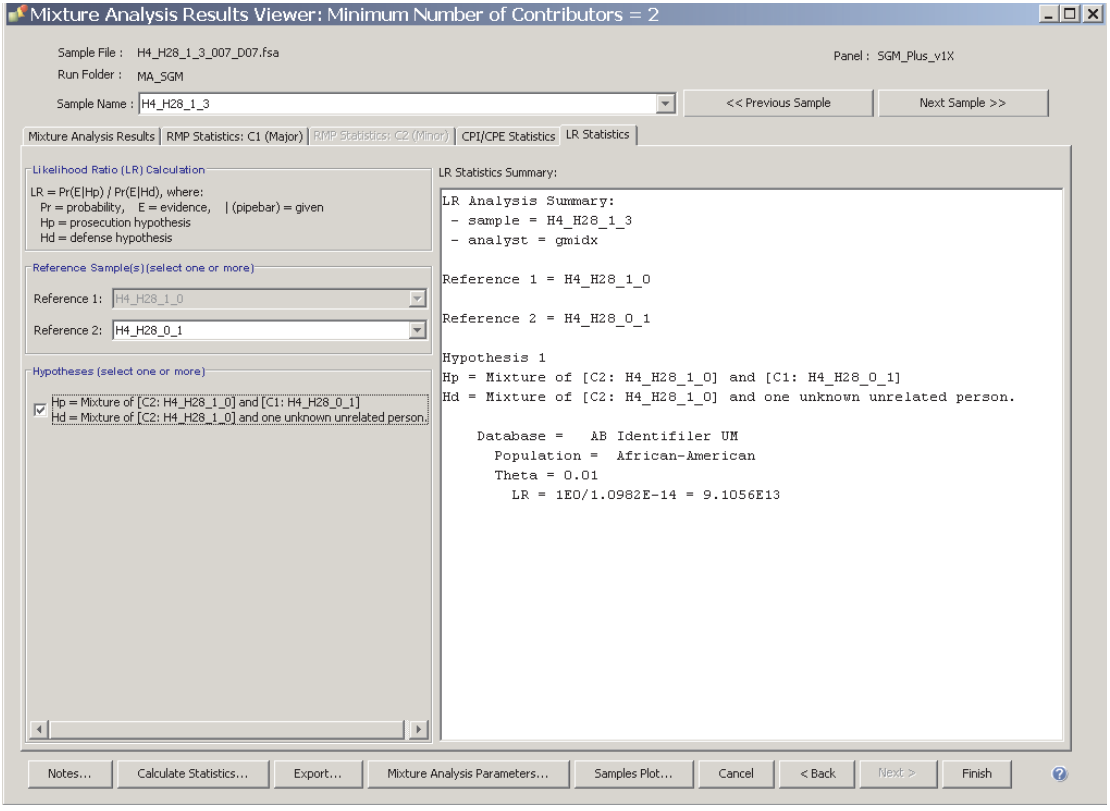


2. Select the known H4_H28_0_1 as Reference 2.

   Only one hypothesis is available.

   - H_p = H4_H28_1_3 originates from a mixture of H4 and H28

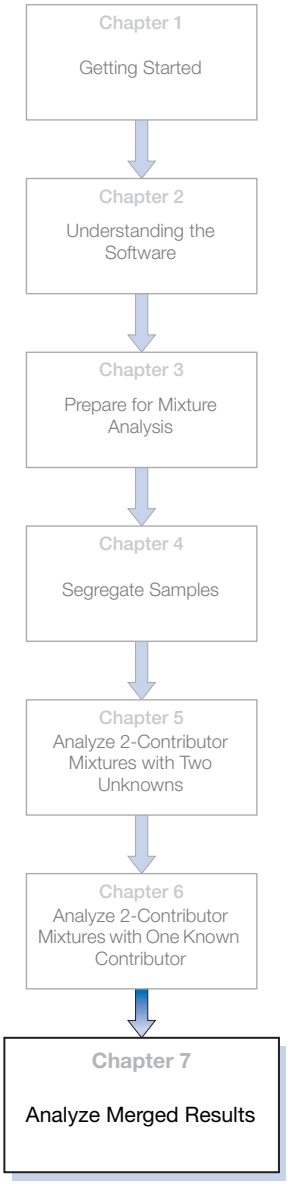
- H_d = H4_H28_1_3 originates from a mixture of H4 and one unknown unrelated person
- The observed profile is 91 trillion times more likely to occur if it originated from a mixture of H4 and one unknown unrelated person.


Mixture Analysis Results Viewer: Minimum Number of Contributors = 2
Sample File : H4_H28_1_3_007_D07.fsa
Panel : SGM_Plus_v1X
Run Folder : MA_SGM
Sample Name : H4_H28_1_3
<< Previous Sample
Next Sample >>
Mixture Analysis Results
RMP Statistics: C1 (Major)
RMP Statistics: C2 (Minor)
CPI/CPE Statistics
LR Statistics
Likelihood Ratio (LR) Calculation
LR = Pr(E|Hp) / Pr(E|Hd), where:
Pr = probability, E = evidence, | (pipebar) = given
Hp = prosecution hypothesis
Hd = defense hypothesis
Reference Sample(s) (select one or more)
Reference 1: H4_H28_1_0
Reference 2: H4_H28_0_1
Hypotheses (select one or more)
Hp = Mixture of [C2: H4_H28_1_0] and [C1: H4_H28_0_1]
Hd = Mixture of [C2: H4_H28_1_0] and one unknown unrelated person.
LR Statistics Summary:
LR Analysis Summary:
- sample = H4_H28_1_3
- analyst = gmidx
Reference 1 = H4_H28_1_0
Reference 2 = H4_H28_0_1
Hypothesis 1
Hp = Mixture of [C2: H4_H28_1_0] and [C1: H4_H28_0_1]
Hd = Mixture of [C2: H4_H28_1_0] and one unknown unrelated person.
Database = AB Identifiler UM
Population = African-American
Theta = 0.01
LR = 1E0/1.0982E-14 = 9.1056E13
Notes...
Calculate Statistics...
Export...
Mixture Analysis Parameters...
Samples Plot...
Cancel
< Back
Next >
Finish

3. Click **Finish**, then **Yes** to exit the Mixture Analysis tool and return to the GeneMapper *ID-X* Project window.

Refer to the *GeneMapper® ID-X Software Help* for additional information on segregating samples, extracting individuals from 2-contributor mixtures, and known matches.

7 Analyze Merged Results



This chapter covers:

- Overview 98
- Combine Different Amplification Results 99
- Step 1: Create a New Project and Add Samples 99
- Step 2: Import an Analysis Method 100
- Step 3: Analyze Samples 100
- Step 4: Prepare for Mixture Analysis 103
- Step 5: Segregate Samples and Review Profiles 104
- Step 6: View Merged Results 105
- Step 7: Calculate and Analyze the Results 106

Overview

This chapter provides guidelines for analyzing merged results. You will learn how to:

- Create a project containing the same sample analyzed with both the AmpFℓSTR® Profiler Plus® and COfiler® kits.
- Add samples to the project.
- Import an analysis method.
- Analyze the samples.
- Prepare for mixture analysis.
- Segregate and review individual profiles.
- Merge the data to create a composite profile containing the 13 genetic markers detectable from both kits.
- Calculate statistics and review results from replicate amplifications of the same sample.

For additional information on replicate amplifications, see the *GeneMapper® ID-X Software Help*.

Combine Different Amplification Results

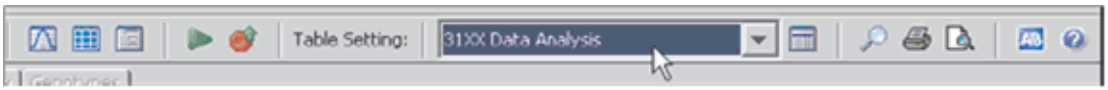
To combine results from different amplifications of the same sample you must create a new project containing the sample files for each amplification of the same sample.

IMPORTANT! Different amplifications of the same sample MUST have the same sample name in order to use the merge function.

The following procedures are based on the sample data provided. See "Using this Guide for Mixture Analysis" on page 6 for additional information.

Step 1: Create a New Project and Add Samples

1. If a project is currently opened, select **File** ▸ **New Project** to open a blank project.
2. If prompted to save changes to an existing project, save any necessary changes or click **No**.
3. In the Project window select **Edit** ▸ **Add Samples to Project** (Ctrl-K).
4. Navigate to *<drive>*:\Example Data\Mixture Analysis Samples, select the **MA_PP** folder, then click **Add to List** in the lower left-hand side of the window to add the sample files to the project.
5. Add the sample files in the MA_CO folder using the same process, then click **Add** when you are finished adding samples to the project list.
6. From the Table Setting drop-down list in the Project window, select 31XX Data Analysis.



Step 2: Import an Analysis Method

Analysis methods define the peak detection, sizing, genotyping, and quality assessment parameters applied during analysis of sample data. In this step, you will import the analysis method configured specifically for use with the GeneMapper® *ID-X* Software example data provided, using the procedures outlined in this guide.

IMPORTANT! The values used in this guide may not be suitable for analyzing data generated in your laboratory. You must optimize and validate these values as part of an internal validation study.

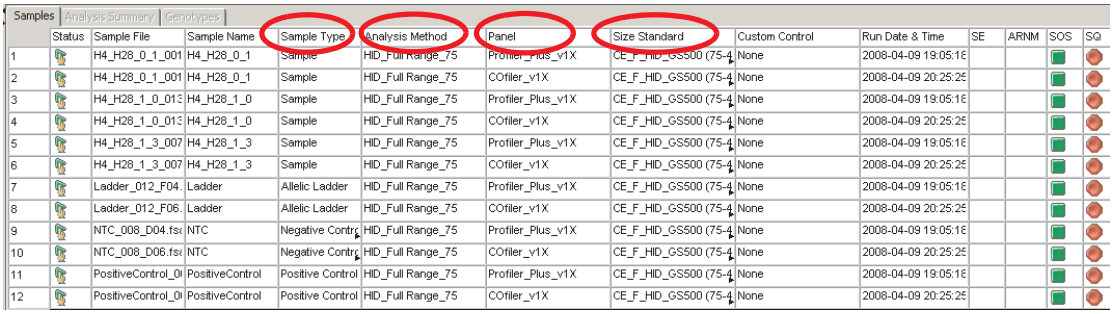
In the GeneMapper *ID-X* Project window:

1. Select **Tools ▸ GeneMapper® ID-X Manager**.
2. In the **Analysis Methods** tab, select **Import**.
3. Navigate to <*drive*>:\AppliedBiosystems\GeneMapper ID-X\Example Data\Mixture Analysis\GSG Analysis Method and select **HID_Full Range_75.**
4. Click **OK** in the Choose Security Group dialog box to confirm the GeneMapper *ID-X* Security Group, then click **Done.**

Step 3: Analyze Samples

1. Define each sample from left to right, as shown in the Samples table below:
   a. Assign the Sample Type to each sample.
   b. Select the Analysis method (HID_Full Range_75).
   c. Select the Panel (Profiler_Plus_v1X or COfiler_v1X).
   d. Select the Size Standard ((CE_F_HID_GS500 (75-450)).

To make your selections, click in each cell and select from the drop-down list. To change an entire column, click in the first cell, make your selection, then click in the column header and press **Ctrl-D** to populate (fill-down) the column.



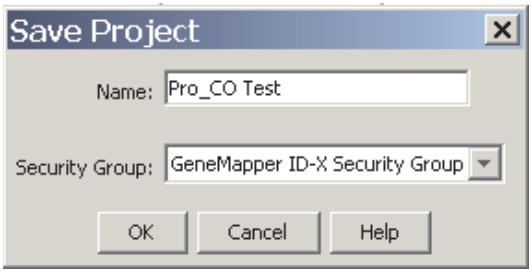
Samples | Analysis Summary | Genotypes

	Status	Sample File	Sample Name	Sample Type	Analysis Method	Panel	Size Standard	Custom Control	Run Date & Time	SE	ARNM	SOS	SQ
1		H4_H28_0_1_001	H4_H28_0_1	Sample	HID_Full Range_75	Profiler_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 19:05:16				
2		H4_H28_0_1_001	H4_H28_0_1	Sample	HID_Full Range_75	COfiler_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 20:25:25				
3		H4_H28_1_0_013	H4_H28_1_0	Sample	HID_Full Range_75	Profiler_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 19:05:16				
4		H4_H28_1_0_013	H4_H28_1_0	Sample	HID_Full Range_75	COfiler_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 20:25:25				
5		H4_H28_1_3_007	H4_H28_1_3	Sample	HID_Full Range_75	Profiler_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 19:05:16				
6		H4_H28_1_3_007	H4_H28_1_3	Sample	HID_Full Range_75	COfiler_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 20:25:25				
7		Ladder_012_F04.	Ladder	Allelic Ladder	HID_Full Range_75	Profiler_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 19:05:16				
8		Ladder_012_F06.	Ladder	Allelic Ladder	HID_Full Range_75	COfiler_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 20:25:25				
9		NTC_008_D04.fsa	NTC	Negative Contr	HID_Full Range_75	Profiler_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 19:05:16				
10		NTC_008_D06.fsa	NTC	Negative Contr	HID_Full Range_75	COfiler_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 20:25:25				
11		PositiveControl_0	PositiveControl	Positive Control	HID_Full Range_75	Profiler_Plus_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 19:05:16				
12		PositiveControl_0	PositiveControl	Positive Control	HID_Full Range_75	COfiler_v1X	CE_F_HID_GS500 (75-4	None	2008-04-09 20:25:25				

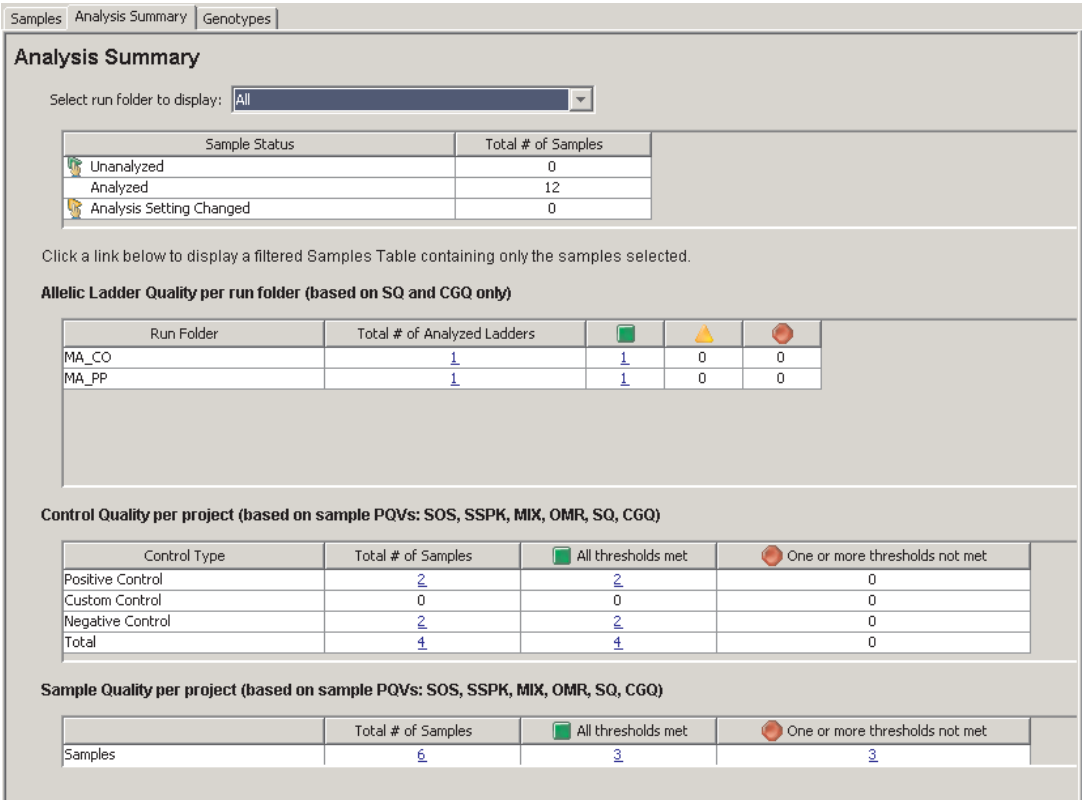
Note: When making changes to the Panel column, a separate window opens instead of a drop-down list. Open the folder and select the appropriate panel.

2. Click the Analyze icon on the toolbar.

3. The Save Project dialog box is displayed. Fill in the Save Project dialog box as shown below, then click **OK**.



The Analysis Summary dialog box is displayed.


Samples
Analysis Summary
Genotypes
Analysis Summary
Select run folder to display: All
Sample Status
Total # of Samples
Unanalyzed 0
Analyzed 12
Analysis Setting Changed 0
Click a link below to display a filtered Samples Table containing only the samples selected.
Allelic Ladder Quality per run folder (based on SQ and CGQ only)
Run Folder
Total # of Analyzed Ladders
MA_CO 1 1 0 0
MA_PP 1 1 0 0
Control Quality per project (based on sample PQVs: SOS, SSPK, MIX, OMR, SQ, CGQ)
Control Type
Total # of Samples
All thresholds met
One or more thresholds not met
Positive Control 2 2 0
Custom Control 0 0 0
Negative Control 2 2 0
Total 4 4 0
Sample Quality per project (based on sample PQVs: SOS, SSPK, MIX, OMR, SQ, CGQ)
Total # of Samples
All thresholds met
One or more thresholds not met
Samples 6 3 3

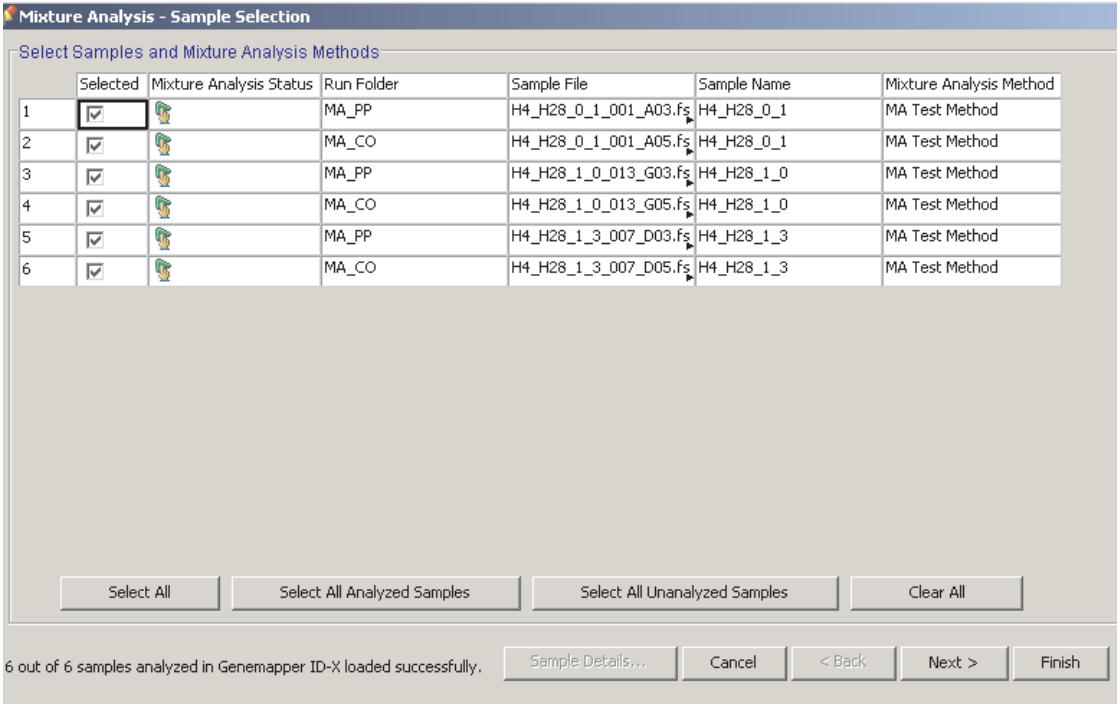
4. In the Analysis Summary under Sample Quality per project, select the 6 samples from the Total # of Samples column to display a filtered samples table that enables edit and review of all the samples.

5. Highlight all sample rows in the table, then click the Display Plots icon in the tool bar to review the data and verify that there are no off-ladder (OL)-only allele labels.

6. Close the Samples Plot.

Step 4: Prepare for Mixture Analysis

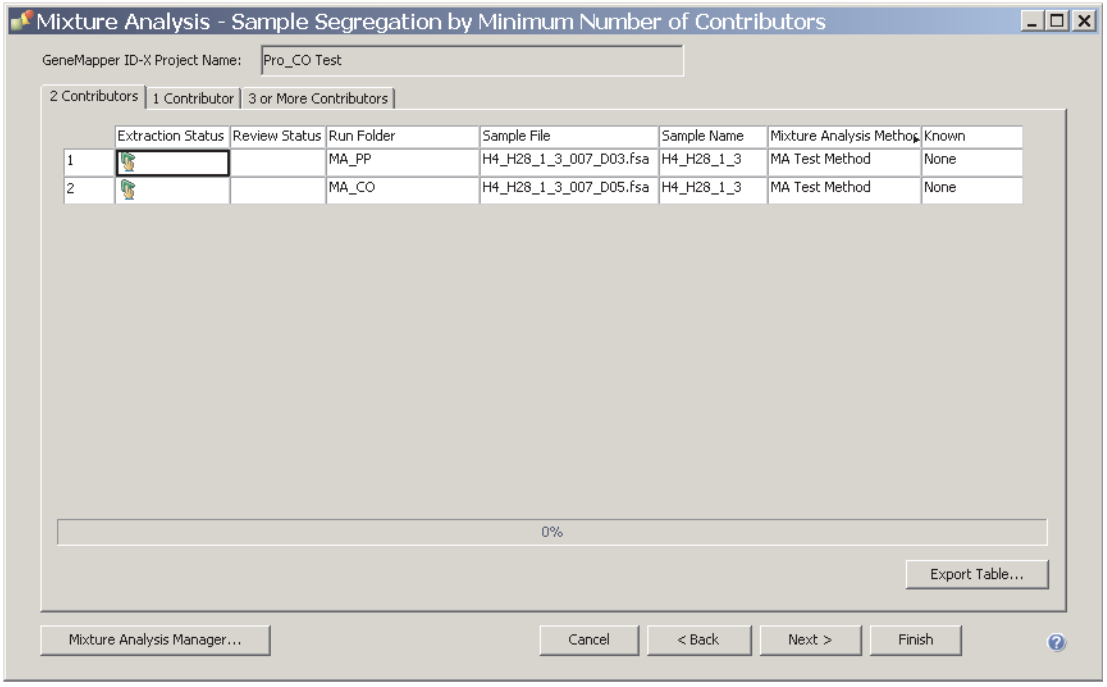
1. In the samples table select **Tools** ▸ **Mixture Analysis**.
2. 1n the Mixture Analysis Method column select **MA Test Method** for all samples and click **Select All**.


Mixture Analysis - Sample Selection
Select Samples and Mixture Analysis Methods
Selected
Mixture Analysis Status
Run Folder
Sample File
Sample Name
Mixture Analysis Method
1 MA_PP H4_H28_0_1_001_A03.fs H4_H28_0_1 MA Test Method
2 MA_CO H4_H28_0_1_001_A05.fs H4_H28_0_1 MA Test Method
3 MA_PP H4_H28_1_0_013_G03.fs H4_H28_1_0 MA Test Method
4 MA_CO H4_H28_1_0_013_G05.fs H4_H28_1_0 MA Test Method
5 MA_PP H4_H28_1_3_007_D03.fs H4_H28_1_3 MA Test Method
6 MA_CO H4_H28_1_3_007_D05.fs H4_H28_1_3 MA Test Method
Select All
Select All Analyzed Samples
Select All Unanalyzed Samples
Clear All
6 out of 6 samples analyzed in Genemapper ID-X loaded successfully.
Sample Details...
Cancel
< Back
Next >
Finish

3. Click **Next>** to proceed with sample segregation as shown in the following section.

Step 5: Segregate Samples and Review Profiles

The two samples segregate as minimum *2 Contributor* mixtures.

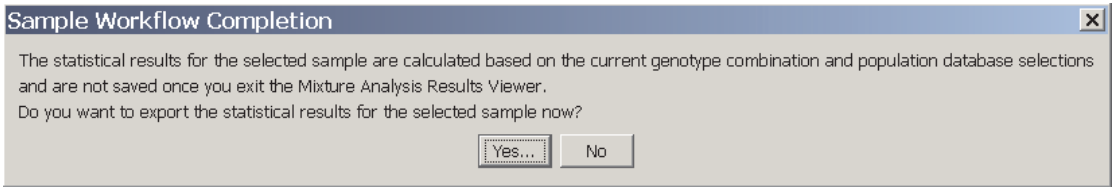


1. In the 2 Contributors tab, click **Next>** to proceed with extraction of contributors.

2. Review the results for sample H4_H28_1_3 analyzed with AmpFℓSTR® Profiler Plus® and COfiler® kits, then check **Sample Review Complete**.

   Note: You will NOT be able to access merged results for samples where the Sample Review Complete box is unchecked.

   The Sample Workflow Completion dialog box is displayed.


Sample Workflow Completion
The statistical results for the selected sample are calculated based on the current genotype combination and population database selections and are not saved once you exit the Mixture Analysis Results Viewer.
Do you want to export the statistical results for the selected sample now?
Yes...
No

3. Click **No.**

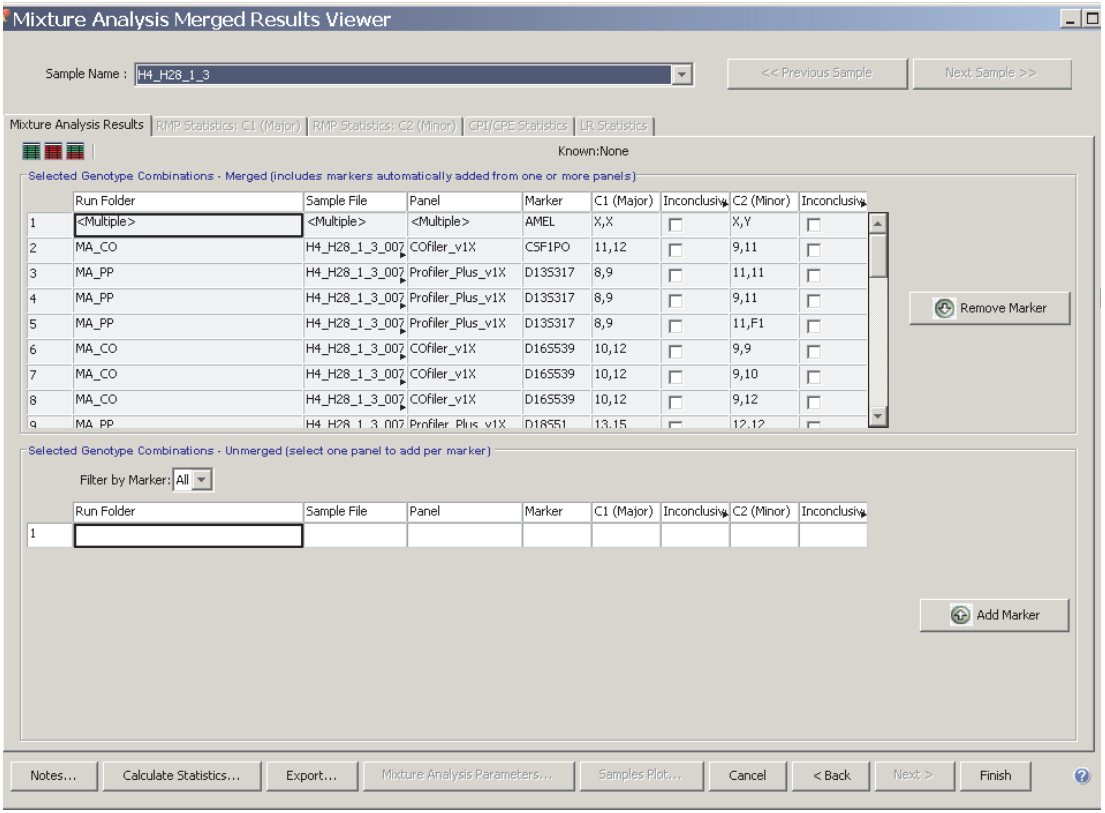
   Note: You will calculate statistics later.

4. Select **Next Sample >>** to review the next sample, repeat procedure steps 3 and 4 above, then proceed to the next section to view the results.

Step 6: View Merged Results

Upon completion of the review of each individual profile, click **Next>** to proceed to the Mixture Analysis Merged Results Viewer and view the mixture analysis results.

The software automatically merges individual results when identical results are obtained for markers that are common to both amplifications, and for instances where only one kit contains a result for a particular marker, as shown in the following figure. This data can be exported to facilitate review.



When you are finished, calculate statistics as shown in the following section.

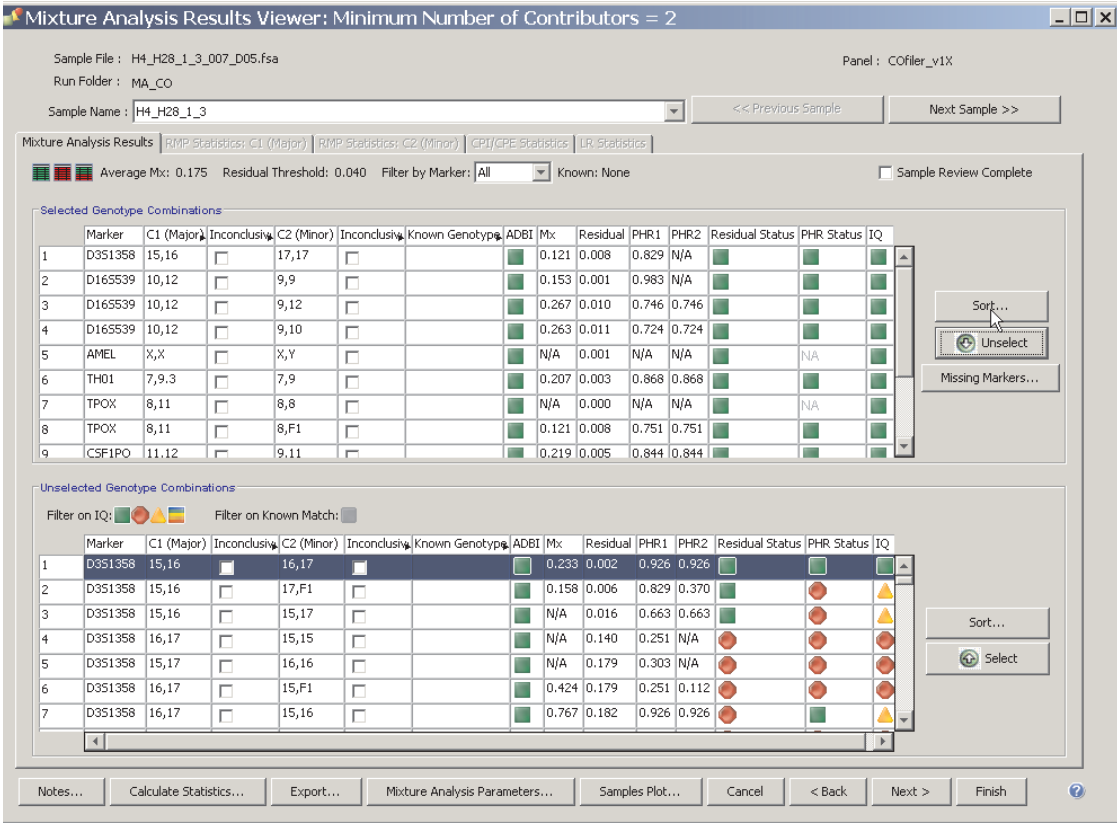
Step 7: Calculate and Analyze the Results

1. Click **Calculate Statistics…**
2. In the Population Database Selection dialog box, select the African-American population for the *Applied Biosystems Identifiler User Manual* database, apply it to the current sample, then click **OK.**
3. Review and analyze the statistics for the composite profile.

4. Click **<Back** to return to the Mixture Analysis Results Viewer displaying the COfiler® kit results.

5. Deselect **Sample Review Complete**.

6. Highlight the result for D3S1358 in Row 1 of the Selected Genotype Combinations table, then click **Unselect** to move the genotype combination to the Unselected Genotype Combinations table as shown below.

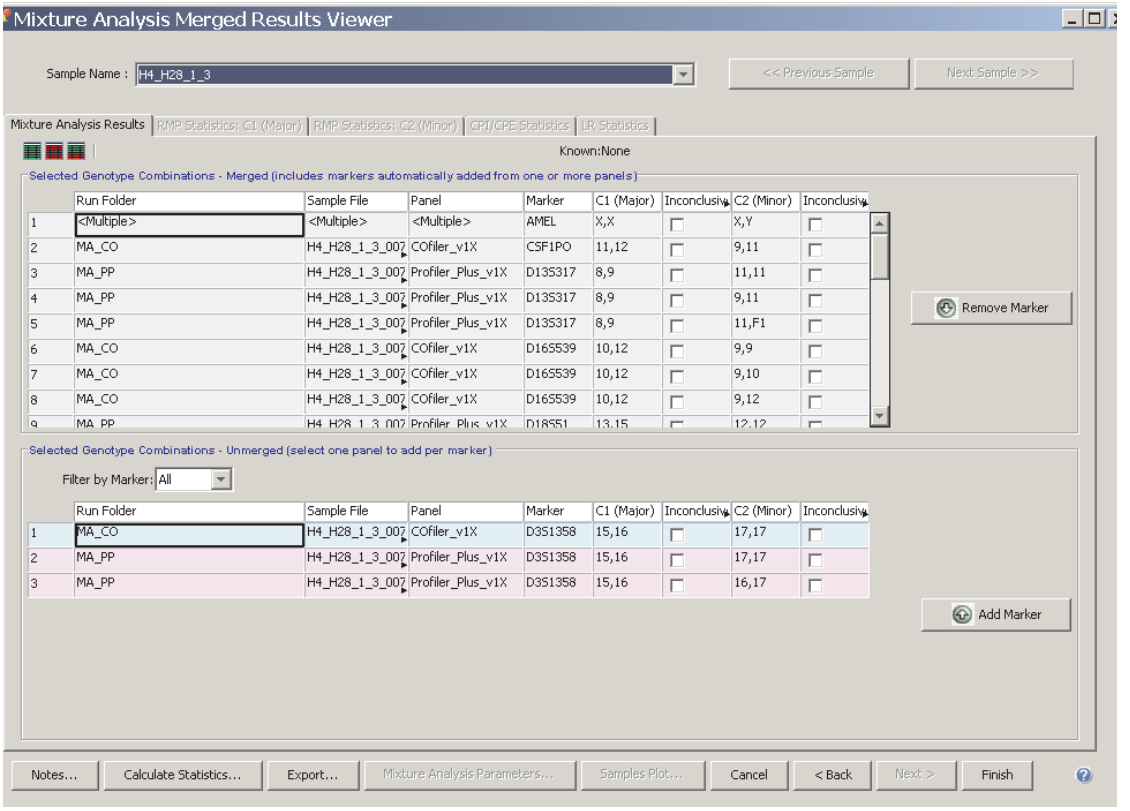

Mixture Analysis Results Viewer: Minimum Number of Contributors = 2
Sample File : H4_H28_1_3_007_D05.fsa
Run Folder : MA_CO
Panel : COfiler_v1X
Sample Name : H4_H28_1_3
<< Previous Sample
Next Sample >>
Mixture Analysis Results
RMP Statistics: C1 (Major)
RMP Statistics: C2 (Minor)
GPI/GPE Statistics
LR Statistics
Average Mx: 0.175
Residual Threshold: 0.040
Filter by Marker: All
Known: None
Sample Review Complete
Selected Genotype Combinations
Marker C1 (Major) Inconclusive C2 (Minor) Inconclusive Known Genotype ADBI Mx Residual PHR1 PHR2 Residual Status PHR Status IQ
1 D3S1358 15,16 17,17 0.121 0.008 0.829 N/A
2 D16S539 10,12 9,9 0.153 0.001 0.983 N/A
3 D16S539 10,12 9,12 0.267 0.010 0.746 0.746
4 D16S539 10,12 9,10 0.263 0.011 0.724 0.724
5 AMEL X,X X,Y N/A 0.001 N/A N/A NA
6 TH01 7,9.3 7,9 0.207 0.003 0.868 0.868
7 TPOX 8,11 8,8 N/A 0.000 N/A N/A NA
8 TPOX 8,11 8,F1 0.121 0.008 0.751 0.751
9 CSF1PO 11,12 9,11 0.219 0.005 0.844 0.844
Sort...
Unselect
Missing Markers...
Unselected Genotype Combinations
Filter on IQ:
Filter on Known Match:
Marker C1 (Major) Inconclusive C2 (Minor) Inconclusive Known Genotype ADBI Mx Residual PHR1 PHR2 Residual Status PHR Status IQ
1 D3S1358 15,16 16,17 0.233 0.002 0.926 0.926
2 D3S1358 15,16 17,F1 0.158 0.006 0.829 0.370
3 D3S1358 15,16 15,17 N/A 0.016 0.663 0.663
4 D3S1358 16,17 15,15 N/A 0.140 0.251 N/A
5 D3S1358 15,17 16,16 N/A 0.179 0.303 N/A
6 D3S1358 16,17 15,F1 0.424 0.179 0.251 0.112
7 D3S1358 16,17 15,16 0.767 0.182 0.926 0.926
Sort...
Select
Notes...
Calculate Statistics...
Export...
Mixture Analysis Parameters...
Samples Plot...
Cancel
< Back
Next >
Finish

7. Mark **Sample Review Complete** and select **Next >**.

8. Click **No** in the Sample Workflow Completion dialog box, then select **Next >**.

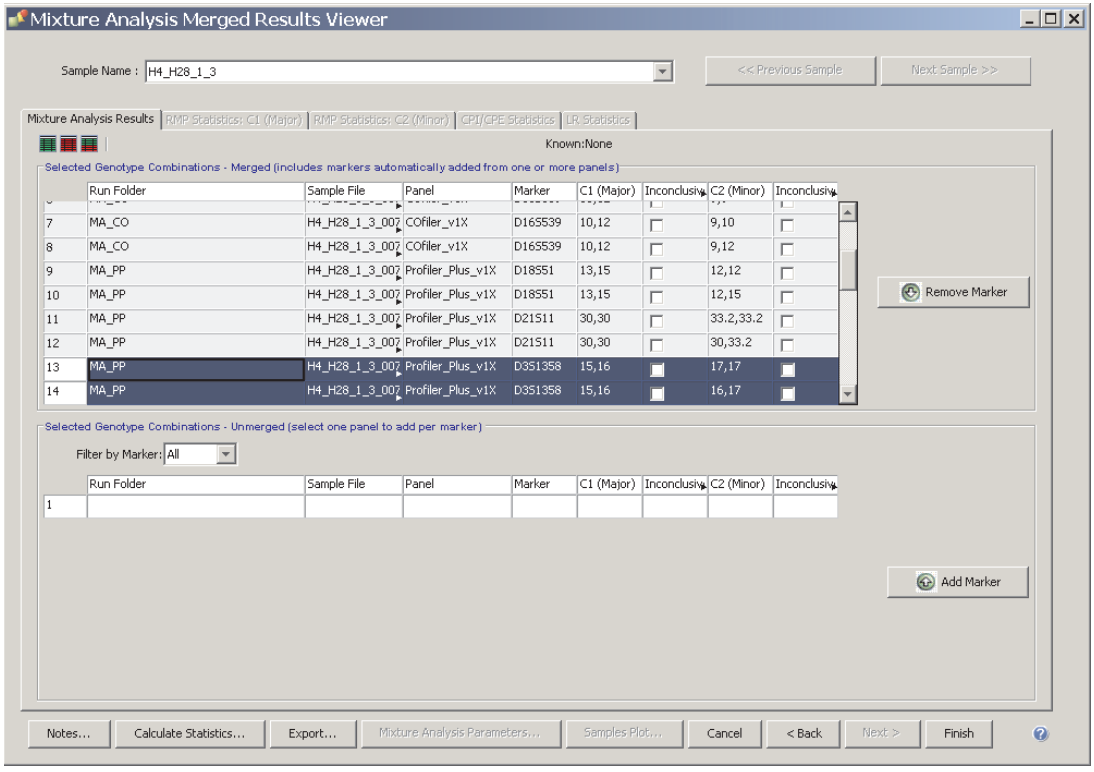
9. View the Mixture Analysis Merged Results Viewer as shown next.

   The Merged Results Viewer now displays the discordant results obtained for D3S1358 using the COfiler® and Profiler Plus® kits.


Mixture Analysis Merged Results Viewer
Sample Name : H4_H28_1_3
<< Previous Sample
Next Sample >>
Mixture Analysis Results
RMP Statistics: C1 (Major)
RMP Statistics: C2 (Minor)
CPI/CPE Statistics
LR Statistics
Known:None
Selected Genotype Combinations - Merged (includes markers automatically added from one or more panels)
Run Folder | Sample File | Panel | Marker | C1 (Major) | Inconclusiv | C2 (Minor) | Inconclusiv
1 <Multiple> <Multiple> <Multiple> AMEL X,X X,Y
2 MA_CO H4_H28_1_3_007 COfiler_v1X CSF1PO 11,12 9,11
3 MA_PP H4_H28_1_3_007 Profiler_Plus_v1X D13S317 8,9 11,11
4 MA_PP H4_H28_1_3_007 Profiler_Plus_v1X D13S317 8,9 9,11
5 MA_PP H4_H28_1_3_007 Profiler_Plus_v1X D13S317 8,9 11,F1
6 MA_CO H4_H28_1_3_007 COfiler_v1X D16S539 10,12 9,9
7 MA_CO H4_H28_1_3_007 COfiler_v1X D16S539 10,12 9,10
8 MA_CO H4_H28_1_3_007 COfiler_v1X D16S539 10,12 9,12
Remove Marker
Selected Genotype Combinations - Unmerged (select one panel to add per marker)
Filter by Marker: All
Run Folder | Sample File | Panel | Marker | C1 (Major) | Inconclusiv | C2 (Minor) | Inconclusiv
1 MA_CO H4_H28_1_3_007 COfiler_v1X D3S1358 15,16 17,17
2 MA_PP H4_H28_1_3_007 Profiler_Plus_v1X D3S1358 15,16 17,17
3 MA_PP H4_H28_1_3_007 Profiler_Plus_v1X D3S1358 15,16 16,17
Add Marker
Notes...
Calculate Statistics...
Export...
Mixture Analysis Parameters...
Samples Plot...
Cancel
< Back
Next >
Finish

10. Select the Profiler Plus® results by highlighting the rows in the bottom table and clicking **Add Marker.**

    The Profiler Plus® results move into the Selected Genotype Combinations - Merged table as shown next.



11. Calculate Statistics *or* click **Finish** to return to the GeneMapper *ID-X* Project window.

Refer to the *GeneMapper® ID-X Software Help* for additional information on analyzing merged results

Statistical Analysis and Algorithms

This appendix covers:

- Overview 112
- Statistical Approaches 113

Overview

This appendix provides supplemental information on:

- Random Match Probability
- Combined Probability of Inclusion/Exclusion
- Likelihood Ratio

Refer to the *GeneMapper® ID-X Software Help* for more detailed information regarding the statistical calculations performed in the software.

Statistical Approaches

The software incorporates three different statistical calculations into the Mixture Analysis Tool: Random Match Probability, Combined Probability of Inclusion/Exclusion, and Likelihood Ratio. All rely on allele frequency data obtained from user-selectable population databases that reside within the software. Analysts can make use of the default population databases supplied with GeneMapper® *ID-X* Software v1.1, or they can import their own population database information to create new selections.

Random Match Probability

The software calculates Random Match Probabilities for those samples categorized as single source and for the individual contributors resulting from two-person mixture extraction. The software computes a minimum allele frequency of $5/2n$, where n = sample number, and uses this minimum allele frequency when the actual allele frequency does not exist in the population database or when the allele frequency is less than the minimum allele frequency.

Homozygous Genotype Frequencies

Homozygous genotype frequencies are calculated as
$(p1 * p1) + p1 * (1.0 - p1) * \theta$

where:

p1= allele 1 frequency

θ = theta correction factor (can be set by the user on a per population basis)

Heterozygous Genotype Frequencies

Heterozygous genotype frequencies are calculated as
$2.0 * (p1 * p2)$

where:

p1 = allele 1 frequency

p2 = allele 2 frequency

In instances of possible allele dropout, the genotype frequency is calculated as $2p$.

In instances of marker dropout (partial profile) or if the marker is deemed inconclusive, the software renders the marker uninformative and substitutes a value of 1.0 for the genotype frequency.

In instances where multiple genotypes are included as possible contributors, the software sums the genotype frequencies at a given marker, resulting in a combined genotype frequency for the marker. It multiplies the combined genotype frequencies to calculate the Random Match Probability for each contributor to the mixture.

Combined Probability of Inclusion/ Exclusion

The software can calculate Combined Probability of Inclusion/Exclusion in instances involving two or more contributors to a mixture.

Probability of Inclusion

The software computes the probability of inclusion for each marker as:

Probability of Inclusion = $(f_1 + f_2 + f_3 + \ldots + f_N)^2$

where:

f_1 = frequency allele 1
f_2 = frequency allele 2
f_3 = frequency allele 3
N= last allele in marker data

Combined Probability of Inclusion

The software computes the combined probability of inclusion as:

Combined Probability of Inclusion = II (Marker Probability of $\text{Inclusion}_{(i)}$)

where:

II = product
i = marker index

For example:

Probability of Inclusion for Marker "D3" = 0.01

Probability of Inclusion for Marker "D5" = 0.025

Combined Probability of Inclusion = [(0.01) * (0.025)] = 0.00025

If the ethnic group = U.S. Hispanic, then:

Combined Probability of Inclusion = 0.00025 for U.S. Hispanic

Stated otherwise, 1 / 0.00025 = 4000 = 1 in 4 thousand U.S. Hispanics

Combined Probability of Exclusion

Combined Probability of Exclusion = 1.0 - Probability of Inclusion

Using the above example:

Combined Probability of Inclusion = 0.00025 for U.S. Hispanic

Combined Probability of Exclusion = 1.0 - 0.00025 = 0.99975

which is expressed as a percentage of the population excluded = 0.99975 * 100 = 99.98%

Likelihood Ratio

Based on available information, the software allows the user to specify a set of hypotheses to calculate Likelihood Ratios for two-person mixtures using the formula:

$LR = Pr\ E|H_p / Pr\ E|H_d$

where:

LR = Likelihood Ratio

Pr = probability

E = evidence

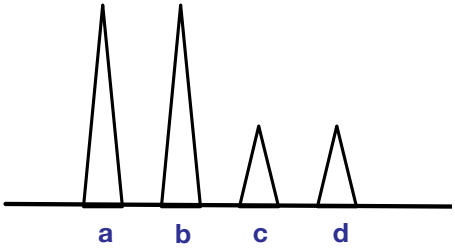
| = given

H_p = Prosecution hypothesis (the explanation put forward by the prosecution for the constituents of the mixture)

H_d = Defense hypothesis (the explanation put forward by the defense for the constituents of the mixture)

Note: H_p and H_d must be mutually exclusive explanations of the mixture evidence.

For example, consider the following mixture scenario originating from an intimate body swab at locus M1:



Assuming a two-person mixture with a suspect of type c, d and a victim of type a, b, the following hypotheses apply:

H_p = The evidence originates from a mixture of the victim and the suspect

H_d= The evidence originates from a mixture of the victim and an unknown person

In this instance:

$H_p = 1$

$H_d = 2p_cp_d$

$LR = 1/2p_cp_d$

The Mixture Analysis Tool provides the following Likelihood Ratio functionality:

- The user may elect to calculate the Likelihood Ratio based on a clear major contributor to the mixture (effectively a single source Likelihood Ratio calculation).
- The user may select one or a multiple set of hypotheses, which are applicable to the two-person mixture data.

- The Likelihood Ratios are calculated based on the extracted data results; that is, only those genotype combinations included in the Selected Genotypes Combinations table will be used in the Likelihood Ratio calculation.

Bibliography

Gill, P. *et al.*, "Interpreting simple STR mixtures using allele peak areas," *Forensic Sci. Int.*, **1998**, *91(1)*, 41-53.

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